AD_____

Award Number: DAMD17-01-2-0056

TITLE: Advanced Cancer Detection Center

PRINCIPAL INVESTIGATOR: Jeffrey P. Krischer, Ph.D.

CONTRACTING ORGANIZATION: University of South Florida
Tampa, Florida 33620

REPORT DATE: October 2003

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release;
Distribution unlimited

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 074-0188

1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	October 2003	Annual (1 Oct 02 - 30 Sep 03)

4. TITLE AND SUBTITLE
Advanced Cancer Detection Center

5. FUNDING NUMBERS
DAMD17-01-2-0056

6. AUTHOR(S)
Jeffrey P. Krischer, Ph.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of South Florida
Tampa, Florida 33620

E-MAIL:
ccontrol@moffitt.usf.edu

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT ***(Maximum 200 Words)***

The goals of the Advanced Cancer Detection Center include the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening and the testing of methods to prevent cancer. The projects included in this report are:

Epoxide hydrolase genetic polymorphisms and their functional significance,

Automated Quantified Screening for Melanoma,

Adaptive Computer Assisted Diagnosis (CAD) Method for Lung Nodule Early Detection,

Breast Cancer Screening in High-Risk Women: Comparison of magnetic resonance imaging (MRI) with mammography,

The Tampa Bay Ovarian Cancer Study, and

Development of the Moffitt Cancer Network.

Each of these projects is presented as a complete study in the attached materials.

14. SUBJECT TERMS
Advanced Cancer Detection

15. NUMBER OF PAGES
135

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

NSN 7540-01-280-5500

Standard Form 298 (Rev. 2-89)
Prescribed by ANSI Std. Z39-18
298-102

Table of Contents

INTRODUCTION:

The **Advanced Cancer Detection Center** (ACDC) of the H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida received initial funding in October 1997. The creation of the Center followed a proposal that was developed in response to legislative language accompanying an appropriation in the Department of Defense budget that appeared in the September 28, 1996, Congressional Record:

"$3,500,000 is available only for the establishment of an advanced cancer detection center for military personnel, dependents, and retired service members, using a network that is in close geographic proximity and includes the following: a military hospital, a regional TRICARE provider, a Department of Veterans Affairs hospital or hospitals, and a medical facility with a focused cancer center that meets the National Cancer Institute eligibility requirements, with respect to research funding. The conferees would expect this center to conduct coordinated screening for cancer detection and treatment, to train military cancer specialists, and to develop improved cancer detection equipment and technology."

Since 1997, the Advanced Cancer Detection Center has received annual funding from the Department of Defense. In 2001, funding that was appropriated in FY00 and FY01 was awarded separately to the H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida. The separate award was made because several projects funded from the original award were still ongoing and funds in the original award were obligated to compete them. Those projects were reported in the final report of DAMD17-98-1-8659. One project, *Development of the Moffitt Cancer Network*, continues beyond the earlier DoD grant and will be part of annual progress reports for the current award, DAMD17-01-2-0056. As additional new projects were and are reviewed and approved, they will be reported on in this progress report.

The ACDC at the H. Lee Moffitt Cancer Center and Research Institute has addressed the goals identified in its appropriations language through studies that target the discovery of molecular and genetic markers of cancer risk, the identification of individuals at high risk for cancer through screening, and the testing of methods to prevent cancer. In addition, the ACDC created and supported education programs to provide increased cancer awareness, provide screening services, and has established working collaborations with the nearby James A. Haley VA Medical Center, the Bay Pines VA Medical Center and the MacDill Air Force Base Hospital. With the success of the Moffitt Cancer Network in providing online cancer education, that project is being expanded to enhance the educational development of the Advanced Cancer Detection Center.

In order to accomplish these goals, the Advanced Cancer Detection Center supports research and demonstration projects that further its mission. An internal peer group and an external scientific advisory committee review each project for scientific merit. Preference is given to projects that have potential to lead to independent peer reviewed funding. During the current grant period, the ACDC supported six cancer prevention and

control research protocols, two of which (the *Development of the Moffitt Cancer Network and The Tampa Bay Ovarian Cancer Study)* were funded under the first Advanced Cancer Detection Award and their funding continued under this award. For this reason they are included in this progress report as well as in the final report of the previous award. The supported studies are:

Epoxide hydrolase genetic polymorphisms and their functional significance,

Automated Quantified Screening for Melanoma,

Breast Cancer Screening in High-Risk Women: Comparison of magnetic resonance imaging (MRI) with mammography, and

Adaptive Computer Assisted Diagnosis (CAD) Method for Lung Nodule Early Detection.

Development of the Moffitt Cancer Network and

The Tampa Bay Ovarian Cancer Study

BODY:

Overview: The H. Lee Cancer Moffitt Center & Research Institute includes a free standing patient care facility with a large inpatient and outpatient capacity, a major research institute consisting of more than 130 scientific members, a free standing Lifetime Cancer Screening Center and a wide array of outreach and educational activities for the general public and select underserved populations. Moffitt Cancer Center's location at the convergence of the University of South Florida's Health Sciences Center and the main campus sets the stage for the its conceptual commitment to interdisciplinary approaches to research and patient care. Moreover, it allows the Center to enjoy all intellectual advantages of a matrix center while remaining operationally freestanding. After 14 years, the Cancer Center's mission remains totally focused on "contributing to the prevention and cure of cancer."

The Cancer Center was created by the Florida Legislature in the early 1980s to meet a clear and compelling need to respond to Florida's "cancer epidemic." Building a major cancer research and treatment center at the University of South Florida in Tampa was largely the vision of H. Lee Moffitt, a state legislator who served as Speaker of the Florida House of Representatives from 1982-84. Construction of the original, 380,000 square foot hospital facility was funded with $70 million from the state's cigarette tax, allowing the Center to open in 1986.

The initial phase of the Cancer Center's strategic plan called for a rapid and substantial deployment of its clinical, financial, and philanthropic resources to develop a true scientific center of excellence. The Center recruited Dr. John C. Ruckdeschel as the

Cancer Center's first director in late 1991. In 1992, he began fulfilling that strategic plan, a process that culminated in the awarding of a Cancer Center Support Grant (CCSG) five years later.

The strategic plan's second phase continues the focus on scientific and clinical growth, with a commitment to increase research facilities by over 200,000 sq.ft., and to prepare to accommodate twice as many patients by 2009. In 1998, the state legislature committed an additional $100 million to finance the construction needed to meet these goals.

In August, 2002, Dr. William Dalton was recruited to become the Cancer Center Director replacing Dr. Jack Ruckdeschel. Dr. Dalton was the Dean of the College of Medicine at the University of Arizona and previously was the Associate Center Director for Clinical Investigations at the Moffitt Cancer Center for 5 years. Thus, Dr. Dalton brings to his new role a considerable experience in the operations of the Cancer Center and an in-depth background in the development of the Cancer Center's scientific agenda.

In April, 2003, Dr. Krischer stepped down as program leader for the Cancer Control Program and returned to the faculty to focus on research. Dr. Thomas Sellers was recruited from Mayo Clinic to be the Associate Center Director for Cancer Control and the new program leader.

Today, the Cancer Center's membership numbers 150 scientists and clinicians who are USF faculty. More than 94 members-in-residence are housed and supported in the Center's facilities and work under the terms of the USF/Moffitt affiliation and faculty support agreements. Other members are based in University departments. The Cancer Center's 1,500 employees support the work of the physicians and scientists. The Center has annual operating revenues of over $130 million yearly, including an $11 million annual appropriation from the State of Florida, research grants totaling more than $36 million overall (direct), philanthropic donations, and institutional commitment from the University of South Florida in the form of faculty salaries and a portion of clinical practice revenues.

The Cancer Center currently supports four scientific programs:

Program	Leader	Members
Molecular Oncology	Richard Jove, Ph.D.	21
Immunology	Julie Djeu, Ph.D.	14
Clinical Investigations	Timothy Yeatman, M.D.	58
Cancer Control	Thomas Sellers, Ph.D.	39
Non-aligned members & institutional grants	N/A	5

The DoD funded Advanced Cancer Detection Center is administratively located with the Cancer Control Research Program. The overall goals of the Cancer Control Research Program remain focused on the reduction of the burden of cancer on individuals and

society. The Cancer Control Research Program is the largest research program at Moffitt with 38 active members and more than $9 million in research funding. The goals of the Cancer Control Research Program are translated into specific focused scientific aims that can be summarized as the application of multidisciplinary research to:

Aim 1	Susceptibility	Identify markers that predict increased cancer susceptibility.
Aim 2	Prevention	Evaluate promising interventions directed at the prevention of cancer.
Aim 3	Early Detection	Develop and test new early detection strategies.
Aim 4	Health Outcomes	Evaluate interventions to improve the quality of life for cancer patients & their care-givers.

On August 23, 2001, the Cancer Control Program held a scientific retreat to discuss the organization and growth of the program, the need for additional core support from the Cancer Center, and opportunities to enhance further intra and inter programmatic collaboration. Subsequently, strategic goals were developed as part of the overall Cancer Center research planning process to coalesce cancer control research into two focus areas. One area included epidemiology and cancer prevention and the other area health outcomes and behavior. Dr. Philip Lazarus was asked to develop the epidemiology and prevention focus and Dr. Paul Jacobsen was asked to develop the health outcomes and behavior focus. Cancer Control investigators were aligned with these two areas based upon their research directions. Aims 1-3 were subsumed into the epidemiology and prevention focus and Aim 4, along with other research targeting behavior changes, was subsumed into the health outcomes and behavior focus. The goals of the alignments were to establish a clear focus and to identify areas for targeted growth. This, in turn, was presented to the Scientific Leadership Council for review and comment and became integrated into the research planning process. The need for new faculty positions, space and infrastructure was identified and included in the Cancer Center budget for this fiscal year. Also, projections were made for additional growth over the next three years.

In order to provide an appropriate mechanism to allocate and manage ACDC funds, the Cancer Center created an administrative core, an internal scientific review committee, and an external advisory committee. The administrative core manages the resources and personnel, associated with the ACDC funding, and provides liaison with the Department of the Army and the regulatory bodies that oversee the research. The internal scientific review committee conducts a scientific review of the merits of proposed projects and their potential for peer-reviewed funding and makes funding recommendations. The external advisory committee reviews the organizational structure and scientific directions of the Advanced Cancer Detection Center and the progress made by the individual projects.

The membership of the internal scientific review committee changes as necessary to have adequate scientific expertise to evaluate proposals submitted to the ACDC. For the fiscal year ending in 2003, the members have been:

Dr. Dmitry Goldgof, Associate Professor, Computer Science and Engineering, College of Engineering
Dr. Pamela Munster, Assistant Professor, Department of Interdisciplinary Oncology, College of Medicine
Dr. Santo Nicosia, Professor and Chair, Department of Pathology, College of Medicine
Dr. Robert Clark, Professor and Chair, Department of Radiology, College of Medicine
Dr. Jeffrey Krischer, ex officio, Professor, Department of Interdisciplinary Oncology, College of Medicine

Cancer Control science at the H. Lee Moffitt Cancer Center and Research Institute is greatly enhanced and facilitated by the development of infrastructure that provides access to shared resources, promotes collaboration and funds pilot projects. Over the last three years, the Cancer Control Program has established new infrastructure to meet these needs. The funding of the Advanced Cancer Detection Center is one of three mechanisms by which this has occurred.

Advanced Cancer Detection Center

The Advanced Cancer Detection Center has become a significant component of the Moffitt Cancer Control Program infrastructure that provides a stimulus for research development and promotes inter and intra programmatic collaborations. The Advanced Cancer Detection Center supports pilot studies that can lead to peer-reviewed extramural funding. Projects supported by this mechanism follow a two-tiered scientific review process in which the science and the likelihood of peer-reviewed extramural funding are considered. In addition, priority is given to projects that foster inter and intra-programmatic collaborations.

Recognizing the great success of this effort, the focus of the Advanced Cancer Detection Center has worked to complement the other infrastructure mechanisms in Cancer Control, most notably the Community Clinical Oncology Program Research Base (described below). That program also provides funds for pilot studies. This has led to the consolidation of the internal advisory committee for each program so that there is continuity between programs. The membership of the consolidated internal advisory committee includes some members from the existing Advanced Cancer Detection Center advisory committee as well as leaders of the Community Clinical Oncology Program Research Base. For 2003-04, the members are:

Dr. Pamela Munster, Assistant Professor, Department of Interdisciplinary Oncology, College of Medicine
Dr. Nagi Kumar, Associate Professor, Department of Interdisciplinary Oncology, College of Medicine
Dr. Rebecca Sutphen, Associate Professor, Department of Interdisciplinary Oncology, College of Medicine
Dr. Jennifer Mayer, Assistant Professor, Department of Pediatrics, College of Medicine

Dr. Paul Jacobsen, Professor, Department of Interdisciplinary Oncology, College of Medicine and Department of Psychology, College of Arts and Sciences
Dr. Jeffrey Krischer, ex officio, Professor, Department of Interdisciplinary Oncology, College of Medicine

These members reflect expertise in genetics, nutrition, behavioral science, endocrinology, oncology, pediatrics and epidemiology. Some have been the principal investigators of studies that have previously received Advanced Cancer Detection Center support and all have experience in obtaining peer-reviewed research support.

In fiscal year 2004, the Advanced Cancer Detection Center will further develop its Telemedicine and Informatics initiatives as a means to further its education objectives contained in enabling legislation. Those technologies, already developed as part of the ongoing Moffitt Cancer Network, will be expanded and further developed to achieve the following objectives:

Task 1: Develop and implement Pediatric Internet Telehomecare Study to assess efficacy of low bandwidth monitoring, management and treatment in the care of childhood chronic diseases.

In conjunction with All Children's Hospital in St. Petersburg Florida, we plan to expand the low-bandwidth video streaming capability developed under the Moffitt Cancer Network to implement a pediatric telemedicine homecare study to assess efficacy of this technology. We hypothesize the use of general monitoring and management devices can greatly improve the transfer of accurate information about the patient's condition to the physician as well as provide the physician a window inside the patient's home to evaluate various complications of his or her disease. We believe the heightened amount of accurate information in addition to remote access to care will improve the ability of the physician and caregiver to care for the patient resulting in overall better care.

Task 2: Develop and implement proof of concept study for genetic counseling delivered from a distance via telemedicine in a multi-center environment.

In conjunction with the Florida Cancer Genetics Network (FCGN), a network of eleven sites providing genetic counseling throughout the state of Florida, we plan to implement a proof of concept study for delivering genetic counseling via telemedicine in a multi-institutional environment. The FCGN is based at the Moffitt Cancer Center and was developed initially under Advanced Cancer Detection Center funding. The Genetics Program at the Moffitt Cancer Center recently concluded a proof of concept for genetic counseling via telemedicine that showed promising results. The proof of concept was designed in such a way as to assess the technology as well as the patient and counselor's resistance to or acceptance of the delivery mode. The patient and counselor were physically located in the same building, although the encounter took place via telemedicine with the use of audio and videoconferencing software.

We propose to extend the scope of the study mentioned above to include multiple centers as well as to assess efficacy using well defined tools to detect differences in knowledge

transfer and patient outcomes relating to overall state of mind post counseling. This extends the current capabilities of the Cancer Network to make scarce resources more widely available to targeted populations and health care providers.

Task 4: Develop and implement an interactive intelligent search and representation system for mining disease information to aid in proper diagnosis.

The system that will be built is a dynamic, self-organizing network of information that will adapt to user needs. When completed, this system will model the data, use machine learning to adapt its own search mechanisms, store its own statistics, and be scalable and 100% dynamic. It will also combine web presentation technologies with analytical systems, require initial education, and be classification and utilization based. There will be a way to add new information into the system, and a way to change how the system learns.

The completed system will dynamically create web pages that display the data that the user has an interest in. It will base its choices on the user's current path and statistical information about relationships, or links between topics. Each user will be able to take a completely different path through the information and find completely different information in the same amount of time.

The more general statement of the problem is to semantically define relationships among granular data elements that reflect a structure imposed on the data by the user. This is equivalent to representing data in a structure such that the user can find related elements without having to know, a priori, the data structure. For example, to be successful in finding a folder that has been filed, the user might be better off knowing the filing system that determines whether the folder has been placed. The filing system might be alphabetical order, subject order, or some other ordering approach. If the filing system is organized by subject, then the user might have to know which is the most closely related subject heading for the file being sought. Yet, the user might have no awareness of how subjects are defined or even named. Similarly, if the task is to retrieve related files, then alphabetical ordering systems provide limited relational groups as compared to subject order filing systems, as long as the definition of the subject groups is explicit. Taken more generally, both data structures require the user to understand the data structure to be successful in any given query. This research will focus on more general data structures that encode relationships and do not require the user to have any prior knowledge. We will explore the application of this approach to the design and construction of web pages, in the context of the Cancer Network, although the problem is much more general.

Moffitt CCOP Research Base (PI:Krischer)

The H. Lee Moffitt Cancer Center received funding by the NCI in June 2000 to develop a research base as a mechanism for Community Clinical Oncology Programs to access cancer control clinical trials. NCI CCOPs and Moffitt affiliates are eligible to participate in the Moffitt CCOP Research Base. Membership is based on continued funding as an NCI CCOP with satisfactory performance measured by accrual and data quality.

The goals of the Moffitt CCOP Research Base are to:

- Develop cancer control trials of high scientific merit for implementation in the community setting.
- Provide community investigators an opportunity to participate in NCI-supported cancer control clinical trials.

The following CCOPs have, or are in the process of, establishing formal affiliations with the Moffitt CCOP research base:
Florida Pediatric CCOP, Tampa, FL
Merit Care Hospital CCOP, Fargo, ND
Mount Sinai Medical Center CCOP, Miami, FL
South Texas Pediatric MBCCOP, San Antonio, TX
Baptist Center Research Institute CCOP, Memphis, TN
Cancer Research for the Ozarks CCOP, Springfield, MO
Columbus CCOP, Columbus, OH
Greater Phoenix CCOP, Phoenix, AZ
North Shore University Hospital CCOP, Manhassett, NY
NorthWest CCOP, Boise, ID
Southern Nevada Cancer Research Foundation CCOP, Las Vegas, NV

The Moffitt CCOP Research Base is now staffed and cancer control protocols and concepts are being initiated. Several of the clinical studies are the result of pilot development funded by ACDC projects. All are approved by the internal advisory committee and then reviewed and approved by the National Cancer Center before activation. Examples of current studies are:

The Specific Role of Isoflavones in Reducing Prostate Cancer Risk	Protocol
A Randomized Pilot Clinical Trial of the Action of Isoflavones and Lycopene in Localized Prostate Cancer: Administration Prior to Radical Prostatectomy.	Protocol
The Effect of Cyproheptadine (periactin) and Megestrol Acetate (Megace) on Weight in Children with Cancer/ Treatment Related Cachexia	Protocol

Cancer Genetic Counseling and Testing by Telemedicine in Community Settings	Concept
Adderall-XR versus Concerta for cancer treatment-related Neurocognitive sequellae and depression in pediatric patients: A randomized phase II study.	Protocol
Stress Management Training for Patients Undergoing Radiotherapy	Protocol
Oral Glutamic Acid to Decrease Vincristine Toxicity in Children with Cancer	Concept
Preservation of Ovarian Function in Young Women Treated with Neoadjuvant chemotherapy for breast cancer: A randomized Trial using the GnRH Agonist (Triptorelin) during adjuvant Chemotherapy	Protocol

Lifetime Cancer Screening and Diagnostic Center

The Lifetime Cancer Screening and Diagnostic Center was administratively realigned during 2001 to be integral to the Cancer Control Program, with the objective of increasing opportunities for cancer control clinical research to be conducted at that site. Now more than a dozen cancer control studies are based at LCS. As well, several of the Advanced Cancer Detection Center approved projects are also based at the Lifetime Cancer Screening and Diagnostic Center. Also, plans have now been made to consolidate cancer control researchers into the Moffitt Research Center as clinics become relocated with the opening of new clinical space. This will provide enhanced opportunities for collaboration as well as improved access to clinical space in which to conduct cancer control studies. The ACDC supported studies in cancer genetics, screening for breast cancer, and screening for lung cancer are all conducted at this site.

(1) KEY RESEARCH ACCOMPLISHMENTS:

The material that follows in this section summarizes the key research accomplishments associated with each project and task outlined in the appropriate approved Statement of Work for ACDC approved projects. **A full description of the projects and their progress is appended.**

Epoxide Hydrolase Genetic Polymorphisms and their Functional Significance.

1. We identified 11 genetic polymorphisms in EH coding region. Among 5 amino acid changing polymorphisms, 2 of them (codons 195 and 382) are previously unidentified.

2. Significant difference of allelic frequencies of EH genetic polymorphisms (codons 113, 139, 450 and intron 4) between African American and Caucasian controls was found (Table 1).

3. We observed significant association between EH genotypes and lung cancer risk in Caucasians (Table 2) (see appendix).

4. We observed significant association between EH genotypes and oralaryngeal cancer risk in Caucasians (Table 3) (see appendix).

Automated Quantified Screening for Melanoma

We have mostly worked on algorithm development and device design. Our analysis has been mostly of parts of the systems and not the system as a whole. Even though our achievements in this stage are mostly on the algorithms that locate and segment the lesions from images, we greatly contributed toward the completion of the system as a whole. We have completed this phase of the research and have results to show the advantage using color correction in our further processing.

Breast Cancer Screening in High-Risk Women: Comparison of Magnetic Resonance Imaging (MRI) with Mammography

To date there are no key research accomplishments. Since we were not given 12 months of enrollment time, we could not complete enrollment of all 300 subjects. Therefore, one-year follow-up report and publication are not feasible.

Adaptive Computer Assisted Diagnosis (CAD) Method For Lung Nodule Detection

PILOT STUDY: CAD vs. Human Accuracy in the Interpretation of Screening Mammograms:

- We have created and validated a CAD algorithm on an independent set of digitized mammograms selected from the VIDI research program
- We have successfully applied the CAD algorithm to a set of 130 cases composed of screening and diagnostic mammograms that represent breast cancer, benign breast disease and normal mammographic features. The initial summary of the performance of CAD with respect to callback rates in screening is provided. (See Appendix)
- We have observed that, at this point in analysis, the CAD performs less accurately than the toss of a fair coin.

The Tampa Bay Ovarian Cancer Study

- Aim 1: Data regarding health behaviors and risk factors were obtained from all participants via questionnaire instruments and study interview was successfully completed on all 231 women. Data regarding menopausal status has been compiled on 138 women. Of the 13 mutation carriers on whom data has been compiled, 0, 4,

and 9 are pre-, peri-, and post-menopausal, respectively. Of the 125 sporadic cases on which data has been compiled, 17, 26, and 82 are pre-, peri-, and post-menopausal, respectively.

- Aim 2: Detailed cancer family history was obtained from all participants via questionnaire instruments and study interview.
- Aim 3: A successful mechanism has been implemented to obtain medical records and tumor tissue in order to compare tumor characteristics between mutation-associated cases and non-mutation controls.
- Aim 4: A successful follow-up mechanism has been implemented to obtain data regarding differences in response to treatment and survival between mutation-associated cases and non-mutation controls.
- Aim 5: Although we were not able to achieve an 80% participation rate, we have been able to accrue a population-based sample with regard to ethnicity, stage, histologic subtype, median age and ethnicity (refer to Tables 2 and 3). Based on Florida Cancer Data System (FCDS) data, we estimate that a total of 430 patients were diagnosed with ovarian cancer during the study period, of whom 350 patients were ascertained (i.e.: 82%). There were 12 patients who died prior to enrollment, 18 patients or doctors declined, and a further 10 patients who could not be enrolled prior to the closing of the study. Hence of the 350 cases ascertained, 231 were enrolled in the study (66%).

Development of the Moffitt Cancer Network

- The Moffitt Cancer Network is available to users and can be found at http://network.moffitt.usf.edu
- The MCN currently has 576 presentations in its library, increasing at a rate of 16 presentations per month on average. Additionally, 16 conferences sponsored by USF and Moffitt are also currently available online.
- All approved Grand Rounds presentations have been taped by the Moffitt Multimedia Education Resources Center (MERC) for over two year preceding this report. The video was previously captured on digital DVCAM 94 minute tapes. Currently we are running in a tape-less environment.
- Since many of the presenters use only 35mm slide for their presentations, a process of creating final production audio/video Real media for streaming via TCP/IP has been developed. This process requires post-production labor and requires the best of the video's individual frames to be captured a second time to recreate higher quality computer images. MCN has made significant progress in this area and as of June 2000 has begun using presenter's PowerPoint files when ever possible to bypass the second image rendering process. This has reduced labor time from 3.5 days to about 5 hours, while increasing image quality noticeably. This labor savings is not realized when presenters are using 35mm film only. This methodology was modified to capture slides, overheads and computer screens digitally without a camera. The new methodology has reduced post-production time to virtually nothing. This allows us to concentrate on acquisition of new material.
- In addition to pre-presentation file acquisition, MCN has begun the development of a presenter packet. When finished, this packet will inform presenters to repeat

important questions asked at the end of events like Grand Rounds and these will be added to the content to be available to medical professionals at the MCN website.

- National oncology conferences have been taped and included in the MCN website database.
- Conferences have been subdivided into their respective presentations and are categorized searchable as well as searchable using the website database Access Jet engine. All conferences are pre-qualified for their ability to become online educational materials by the University of South Florida College of Medicine and, more recently, the University of South Florida College of Nursing.
- MCN began simultaneous live streaming and archiving in late 2001. This process greatly reduces postproduction time while increasing access to live events.
- MCN has completed the move to camera-less and tape-less acquisition of presentations using a host of digital equipment.

(2) REPORTABLE OUTCOMES:

Manuscripts, abstracts, presentations:

Epoxide Hydrolase Genetic Polymorphisms and their Functional Significance.

Manuscript related to this study

1. An article was published in Oral Oncology (attached to appendix)
   Park, J., Schantz SP and Lazarus, P. (2003) Epoxide hydrolase genotype and orolaryngeal cancer risk: Interaction with GSTM1 genotype. Oral Oncology 39:(5) 483-490.

2. A manuscript was accepted. (attached to appendix)
   Chen, L., Tockman M, Elahi, A., Lazarus, P., and **Park. J.** (2004). Genetic analysis of microsomal epoxide hydrolase gene and its association with lung cancer risk. (Accepted International Journal of Cancer. 2004)

Abstracts

Results of these studies supported by this award were presented at three meetings (1 oral, 2 poster presentations). The abstracts for poster presentation were attached to appendix section.

1. **Park, J.,** and Lazarus, P (2001) Epoxide hydrolase polymorphism and oral cancer risk: correlation with the GSTM1-null genotype. Proc. Amer. Assoc. cancer res. 40:566, 2001. The 92nd Annual Meeting American Association for Cancer Research. March 24-28, 2001. New Orleans, LA.

2. Chen, L., Tockman, M. and **Park, J.** (2002) Epoxide hydrolase polymorphisms and risk for lung cancer. NIH/NCI/Early detection research network (EDRN). The 5th Steering Committee Meeting. Oral presentation, February 3-5, 2002. M. D. Anderson Cancer Center, Houston, TX

3. Chen, L., Lazarus, P., Tockman, M. and **Park, J.** (2002) Epoxide hydrolase polymorphisms and risk for lung and oral cancer. Gordon Research conferences. Poster presentation, New Frontiers in Cancer Detection & Diagnosis. March 10-15, 2002. Ventura, CA

Funding applied for based on work supported by this award:

Plan to resubmit R01 (R01CA104270)

Title: Genetic risk factors for lung cancer

PI: Jong Park

The major goal of this project is to investigate genetic polymorphisms may determine individual lung cancer susceptibility.

Employment supported by this award:

This award support the following personnel:

PI (Jong Park),

Lan Chen (MS, Research Assistant),

Kristin Shade (MPH, Research Assistant),

Kun Li (BS, Research Assistant).

Automated Quantified Screening for Melanoma

- Yelena Mukomel, who has worked on the design of the computer algorithms, received her Master's degree in October 2002.
- We were successful in getting an equipment grant from the National Science Foundation (NSF Grant EIA 0130768) to buy the Minolta range scanner that will be used in this study.
- Krassimir Ivanov, who has worked on the design of the computer algorithms, received his Master's degree in October 2002.
- All the necessary hardware and software has been acquired and developed

Breast Cancer Screening in High-Risk Women: Comparison of Magnetic Resonance Imaging (MRI) with Mammography

To date no reportable outcomes have resulted from this research. Since we were not given 12 months of enrollment time, we could not complete enrollment of all 300 subjects. Therefore, one-year follow-up, report, and publication are not feasible.

Adaptive Computer Assisted Diagnosis (CAD) Method For Lung Nodule Detection

Funding applied for based on work supported by this award:

Plans are underway to submit a competing continuation of R01CA74110 based on the analysis of the performance of CAD, March 1, 2004.

The Tampa Bay Ovarian Cancer Study

Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study, funding was awarded by the American Cancer Society for a companion study to evaluate the role of biologically active lysophospholipids for their potential as biomarkers of ovarian cancer (7/1/00 – 6/30/04). Preliminary data is promising and shows that certain lysophospholipids appear to be elevated in the plasma of women with ovarian cancer compared with healthy controls (article accepted in Cancer Epidemiology, Biomarkers, and Prevention and included in Appendix A). We have applied to ACS for a two-year extension of the project. Also, based on this preliminary data, we have applied to NIH for R01 funding to investigate the use of lysophospholipid measurement and proteomic profiles for detection of ovarian cancer in a case-control study.

Based on data showing that gene mutations associated with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) are the third leading cause of hereditary ovarian cancer (after BRCA1 and BRCA2), and the suggestion that ovarian cancer is a "sentinel cancer" in individuals with these gene mutations, an investigation of HNPCC as a companion study of TBOCS has been funded.

The preliminary results of this research were presented at the 2002 American Society of Human Genetics annual meeting (Appendix B), and 2003 Frontiers in Cancer Prevention Research (American Association for Cancer Research) annual meeting (Appendix C).

Development of the Moffitt Cancer Network

Abstracts Related to this Study:

- J Permuth-Wey, JA Betts, AB Cantor, JP Krischer, R. Sutphen: Cancer Genetic Counseling and Testing by Telemedicine - Results of a Feasibility Study (Abstract). American Journal of Human Genetics (2002) 71(4): 343.

Presentations Related to this Study:

- The Moffitt Cancer Network Vision, Jeffery Krischer, Ph.D. April 2001
- The Moffitt Cancer Network, Lessons Learned and New Directions, Matthew Clark, B.S. October 2001
- The Moffitt Cancer Network 2002, Matthew Clark, B.S. April 2002
- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., May 19, 2002 Medical Library Association Conference, Dallas TX
- No-latency video architecture, efficiency and a new tomorrow for on-line education, Matthew Clark, B.S. June 2002

- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., June 19, 2002 Tech Topics, Moffitt
- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., October 19, 2002 Southern Chapter, Medical Library Association
- Disseminating Library Instruction to the Desktop via the Web, Sue Felber, M.S., October 19, 2002 Southern Chapter, Medical Library Association
- Telemedicine Today and Tomorrow, Matthew Clark, B.S. October 2002

- Patents and licenses applied for and/or issued:

  A notice of disclosure has been filed with the USF office of patents in anticipation of the completion of a patent application.

- Funding received based on work supported by this award:

  The technologies that have been developed under the auspices of the Advanced Cancer Detection Center have led to several grant proposals and many are still pending. Two large grants that have incorporated the technology of the Moffitt Cancer Network into data coordinating center applications that have recently been funded to the Moffitt are:

  The Data and Technology Coordinating Center for the NIH Rare Disease Network (PI: Jeffrey Krischer, Ph.D.)

  and

  The Data Coordinating Center for the Study of the Environmental Determinants of Diabetes in the Young. (PI: Jeffrey Krischer, Ph.D.)

(3) CONCLUSIONS:

The Advanced Cancer Detection Center continues to be successful. Some projects originated under the previous funding (DAMD17-98-1-8659) have been completed under the auspices of this award and others are continuing. The research has led to publications, presentations and successful grant applications. All projects have been approved for human subjects both at the University of South Florida Institutional Review Board and at the DoD Human Subjects Review Committee.

The Advanced Cancer Detection Center has been successful in developing and implementing a variety of bleeding edge technologies over the past five years. We plan to continue developing new technologies as well as extending existing technologies that contribute to the improvement in quality of overall patient care and public health.

(4) REFERENCES:

References pertinent to the individual projects are contained in the appended material.

APPENDIX A

Epoxide Hydrolase Genetic Polymorphisms and Their Functional Significance

Jong Park, PhD

Final Report

Proposal Title: Epoxide hydrolase genetic polymorphisms and their functional significance.

PI: Jong Y. Park, PhD

INTRODUCTION:

Genetic variations in pathways involved in the metabolic activation and detoxification of tobacco carcinogen are likely to be a major source of inter-individual variation in cancer susceptibility. One of the important enzymes involved in the metabolism of major tobacco-smoke carcinogens including polycyclic aromatic hydrocarbons (PAHs) like benzo[a]pyrene (BaP) is epoxide hydrolase (EH). Previous studies have suggested EH polymorphisms in increased risk for smoking related cancers including both lung as well as oral cancer patients. In preliminary studies, we have identified two new genetic polymorphisms present in the coding regions of the corresponding EH protein. These polymorphisms, present in codons 43 and 382 of the EH gene, results in amino acid changes from arginine to threonine for codon 43, and from tryptophan to leucine for codon 382. Our hypothesis is that these newly-identified genetic polymorphisms may play an important role in risk for tobacco-related cancers. In this proposal, we propose to assess the functional significance of newly-identified polymorphisms in terms of their ability to affect EH-catalyzed metabolism of benzo[a]pyrene-7,8-epoxide to the corresponding diol. In addition we also propose to investigate correlation between EH genotype and phenotype and role in the risk for smoking related cancers.

BODY:

As a part of proposed study, we screened previously unidentified polymorphisms in EH gene using three different detecting methods (Expressed sequence tag data search, single strand conformational polymorphism, and direct sequencing). Table 1 shows the location and characters of 11 polymorphisms identified in the samples we analyzed. Among the 11 polymorphisms detected, five polymorphisms resulted in either amino acid substitutions, or silent, respectively, and a polymorphism was located in the intron 4 region. Among the five amino acid changing polymorphisms, three of them were identified in previous studies (1, 2, 3). The allelic frequencies of codon 113 and 139 polymorphisms were similar to those observed in previous studies of both Caucasians and African Americans (2, 4-7). The allelic frequency for codon 43 polymorphism was reported as 0.08 in a small study (1) (n=26), but we observed significantly lower allelic frequency in our total control population (0.01, n=252).

Differences in EH allelic frequencies were observed between Caucasians and African American controls. Two polymorphisms, which are located in intron 4 ($p < 0.02$) and at codon 450 ($p < 0.01$) show significantly different allelic frequencies between two control groups. In addition, a significantly ($p < 0.0001$) lower frequency of the EH^{139Arg} allele was observed for Caucasians (0.15) as compared to African Americans (0.31), while the EH^{113His} allelic frequency was significantly ($p < 0.0001$) higher in Caucasians (0.40) as compared to African Americans (0.20; Table 1).

Table 1. Prevalences of polymorphisms in EH gene in Caucasian and African American controls

Location	Codon	Sequence change	Amino acid change	Allelic frequencies	
				Caucasians	African Americans
Exon 2	43[1]	G<C	Arg<Thr	0.01 (4/336)[2]	0.01 (2/168)
Exon 3	113[1]	T<C	Tyr<His	0.40 (136/336)[3]	0.20 (54/274)
Exon 3	119	G<A	Silent (Lys)	0.13 (25/188)	0.16 (16/100)
Intron 4	+34[1]	G<A		0.10 (16/166)[3]	0.02 (2/102)
Exon 4	139[1]	A<G	His<Arg	0.15 (50/334)[3]	0.31 (89/286)
Exon 4	149	C<T	Silent (Gly)	0.01 (2/166)	0.01 (1/100)
Exon 4	195	T<C	Ser<Pro	0.006 (1/168)	0.01 (1/102)
Exon 6	284	C<T	Silent (Pro)	0.08 (11/146)	0.08 (9/108)
Exon 8	357	T<C	Silent (Asn)	0.11(18/168)	0.14(20/142)
Exon 8	382	G<T	Trp<Leu	0.006 (1/168)	0.01 (1/100)
Exon 9	450	G<C	Silent (Ser)	0.06(10/168)[3]	0(0/100)

[1] Previously reported polymorphisms
[2]Assay sample size (number of alleles detected/number of alleles screened).
[3]Allelic frequencies were significantly different between African American and Caucasian controls.

As a part of Task 1, we obtained vector containing entire EH gene from collaborator (Dr. C. Omiecinski, University of Washington). We created plasmids containing new amino acid changing polymorphisms (codons 43, 195, and 382) identified in our laboratory by using site–directed mutagenesis. However, in-vitro functional analysis of these plasmids

was not performed because allelic frequencies of these polymorphisms were too low (0.01) to affect risk for lung cancer in general population.

As a part of Task 2, we performed PCR-relative fragment length polymorphisms (RFLP) for genotyping of two functionally related, amino acid changing polymorphisms (codons 113 and 139). We compared prevalence of these polymorphisms in Caucasians and African Americans controls (Table 1), and assessed role in risk for smoking related lung cancer (Table 2).

Table 2. Demographic information of subjects, EH polymorphisms, predicted EH activity genotypes and lung cancer risk.

			Cases	Controls	
n			169	169	
mean age (range)			65.2 (36-83)	61.7 (31-86)	
Sex (M/F)			100/69	100/69	
Smoking [mean ± SD (py)][1]			53.1 ± 31.7	18.8 ± 25.0	
Location	Codon	genotypes	Cases	Controls	OR (95%CI)[2]
Exon 2	43	arg/arg	169 (100)[3]	164 (98)	1.0 (referent)
		arg/thr	0 (0)	4 (2)	NA[4]
Exon 3	113	his/his	25 (15)	38 (23)	1.0 (referent)
		tyr/his	67 (41)	60 (36)	2.8 (1.3-6.1)
		tyr/tyr	77 (44)	70 (41)	2.4 (1.1-5.0)
Exon 4	139	his/his	115 (68)	120 (72)	1.0 (referent)
		arg/his	47 (28)	44 (26)	1.6 (0.9-2.8)
		arg/arg	7 (4)	3 (2)	2.4 (0.5-11.4)
Predicted EH activity genotypes[5]			Cases	Controls	OR (95%CI)[2]
low			20 (12)	32 (19)	1.0 (referent)[6]
intermediate			117 (69)	114 (69)	2.7 (1.3-5.9)
high			32 (19)	20 (12)	5.0 (1.9-13.3)

[1] Smoking consumption was significantly higher ($p < 0.001$) in cases as compared to controls.
[2] ORs were calculated by adjusting for sex, age, and smoking (py).
[3] Numbers in parenthesis denote percentages.
[4] p=0.061 by Fisher exact test.
[5] (high), subjects with either the EH*1/EH*2, or EH*2/EH*2 genotypes; (intermediate), subjects with either the EH*1/EH*1, EH*1/EH*3, EH*2/EH*3, EH*2/EH*4 or EH*3/EH*4 genotypes; (low), subjects with either the EH*3/EH*3 genotype.
[6] Significant increase in predicted high-risk genotypes as determined by χ^2-trend test ($p = 0.018$).

To investigate association between EH polymorphisms and oral cnacer risk, we performed case:control studies for the tobacco-related, oral cancer (Table 3) in Caucasians and African Americans. Results of these studies were published in Oral Oncology (see appendix)

Table 3. EH polymorphic variants and orolaryngeal cancer risk in Caucasians and African Americans.

EH polymorphisms	Caucasians cases	Caucasians controls	Caucasians OR (95%CI)[a,b]	African Americans cases	African Americans controls	African Americans OR (95%CI)[a,c]
113his/his	19 (13)[d]	50 (23)	1.0 (referent)	4 (5)	9 (7)	1.0 (referent)
113tyr/his+tyr/tyr	123 (87)	163 (77)	2.1 (1.1-4.0)	77 (95)	113 (93)	2.4 (0.5-12.2)
139his/his	86 (61)	144 (68)	1.0 (referent)	31 (38)	60 (49)	1.0 (referent)
139arg/his +arg/arg	56 (39)	69 (32)	1.3 (0.8-2.2)	50 (62)	62 (51)	1.3 (0.6-2.7)

[a] ORs were calculated by adjusting for sex, age, smoking (py), alcohol consumption (categorical variables), and region of subject recruitment.
[b] Nine or [c]three subjects were excluded from OR calculations due to incomplete questionnaire data.
[d] Numbers in parenthesis denote percentages.

KEY RESEARCH ACCOMPLISHMENTS:

1. We identified 11 genetic polymorphisms in EH coding region. Among 5 amino acid changing polymorphisms, 2 of them (codons 195 and 382) are previously unidentified.

2. Significant difference of allelic frequencies of EH genetic polymorphisms (codons 113, 139, 450 and intron 4) between African American and Caucasian controls was found (Table 1).

3. We observed significant association between EH genotypes and lung cancer risk in Caucasians (Table 2) (see appendix).

4. We observed significant association between EH genotypes and oralaryngeal cancer risk in Caucasians (Table 3) (see appendix).

REPORTABLE OUTCOMES:

Manuscript

1. An article was published in Oral Oncology (attached to appendix)
   Park, J., Schantz SP and Lazarus, P. (2003) Epoxide hydrolase genotype and orolaryngeal cancer risk: Interaction with GSTM1 genotype. Oral Oncology 39:(5) 483-490.

2. A manuscript was accepted. (attached to appendix)

   Chen, L., Tockman M, Elahi, A., Lazarus, P., and **Park. J.** (2004). Genetic analysis of microsomal epoxide hydrolase gene and its association with lung cancer risk. (Accepted International Journal of Cancer. 2004)

Abstracts

Results of these studies supported by this award were presented at three meetings (1 oral, 2 poster presentation). The abstracts for poster presentation were attached to appendix section.

1. **Park, J.,** and Lazarus, P (2001) Epoxide hydrolase polymorphism and oral cancer risk: correlation with the GSTM1-null genotype. Proc. Amer. Assoc. cancer res. 40:566, 2001. The 92nd Annual Meeting American Association for Cancer Research. March 24-28, 2001. New Orleans, LA.

2. Chen, L., Tockman, M. and **Park, J.** (2002) Epoxide hydrolase polymorphisms and risk for lung cancer. NIH/NCI/Early detection research network (EDRN). The 5th

Steering Committee Meeting. Oral presentation, February 3-5, 2002. M. D. Anderson Cancer Center, Houston, TX

3. Chen, L., Lazarus, P., Tockman, M. and **Park, J.** (2002) Epoxide hydrolase polymorphisms and risk for lung and oral cancer. Gordon Research conferences. Poster presentation, New Frontiers in Cancer Detection & Diagnosis. March 10-15, 2002. Ventura, CA

Funding applied for based on work supported by this award:

Plan to resubmit R01 (R01CA104270)

Title: Genetic risk factors for lung cancer

PI: Jong Park

The major goal of this project is to investigate genetic polymorphisms may determine individual lung cancer susceptibility.

Employment supported by this award.

This award support following personnel:

PI (Jong Park),

Lan Chen (MS, Research Assistant),

Kristin Shade (MPH, Research Assistant),

Kun Li (BS, Research Assistant).

CONCLUSIONS:

In this proposed studies, we have found new polymorphisms in EH coding regions and found important role in risk for smoking related cancers.

We demonstrated combined genotypes with epoxide hydorlase and glutathione S transferase (GSTM1), involved in detoxification of BaP-7, 8-epoxide, affect on smoking related cancer susceptibility We observed a significant association between amino acid changing EH polymorphisms (codons 113 and 139) and risk for oralaryngeal, and lung cancers in Caucasians. This association is smoking dose-dependent, with significantly increased risk observed for Caucasian subjects with predicted high EH activity genotypes who were smokers. These results are consistent with a critical role for EH in tobacco-related cancer risk and in the metabolism of BaP-7, 8-epoxide to BaP-7,8-dihydrodiol.

In summary, polymorphisms of EH gene appear to play an important role in susceptibility to oralaryngeal and lung cancers, with a smoking dose-dependent association.

REFERENCES:

1. Gaedigk A, Spielberg SP, Grant DM. Characterization of the microsomal epoxide hydrolase gene in patients with anticonvulsant adverse drug reactions. *Pharmacogenetics* 1994;4(3):142-53.

2.. Green VJ, Pirmohamed M, Kitteringham NR, Gaedigk A, Grant DM, Boxer M, Burchell B, Park BK. Genetic analysis of microsomal epoxide hydrolase in patients with carbamazepine hypersensitivity. *Biochem Pharmacol* 1995;50(9):1353-9.

3.. Hassett C, Aicher L, Sidhu JS, Omiecinski CJ. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. *Hum Mol Genet* 1994;3(3):421-8.

4. London SJ, Smart J, Daly AK. Lung cancer risk in relation to genetic polymorphisms of microsomal epoxide hydrolase among African-Americans and Caucasians in Los Angeles County. *Lung Cancer* 2000;28(2):147-55.

5. Wu X, Gwyn K, Amos CI, Makan N, Hong WK, Spitz MR. The association of microsomal epoxide hydrolase polymorphisms and lung cancer risk in African-Americans and Mexican-Americans. *Carcinogenesis* 2001;22(6):923-8.

6. Benhamou S, Reinikainen M, Bouchardy C, Dayer P, Hirvonen A. Association between lung cancer and microsomal epoxide hydrolase genotypes. *Cancer Res* 1998;58(23):5291-3.

7. Jourenkova-Mironova N, Mitrunen K, Bouchardy C, Dayer P, Benhamou S, Hirvonen A. High-activity microsomal epoxide hydrolase genotypes and the risk of oral, pharynx, and larynx cancers. *Cancer Res* 2000;60(3):534-6.

APPENDICES:

1. PI's Curriculum Vitae

2. A copy of an article that was published in Oral Oncology
   Park, J., Schantz SP and Lazarus, P. (2003) Epoxide hydrolase genotype and orolaryngeal cancer risk: Interaction with GSTM1 genotype. Oral Oncology 39:(5) 483-490.

3. A manuscript that was accepted. (Attached to appendix)
   Chen, L., Tockman M, Elahi, A., Lazarus, P., and **Park. J.** (2004). Genetic analysis of microsomal epoxide hydrolase gene and its association with lung cancer risk. (Accepted International Journal of Cancer. 2004)

4. A copy of abstract presented at the annual AACR meeting
   Park, J., and Lazarus, P (2001) Epoxide hydrolase polymorphism and oral cancer risk: correlation with the GSTM1-null genotype. Proc. Amer. Assoc. cancer res. 40:566, 2001. The 92nd Annual Meeting American Association for Cancer Research. March 24-28, 2001. New Orleans, LA.

5. A copy of abstract presented at the Gordon conferences
   Chen, L., Lazarus, P., Tockman, M. and **Park, J.** (2002) Epoxide hydrolase polymorphisms and risk for lung and oral cancer. Gordon Research conferences. Poster presentation, New Frontiers in Cancer Detection & Diagnosis. March 10-15, 2002. Ventura, CA

CURRICULUM VITAE

Jong Y. Park, DrPH

Education

1995 DrPH
Department of Epidemiology and International Public Health.
School of Public Health, University of Alabama at Birmingham. Birmingham, AL.
"Prevalence of Infection with Lymphocytic Choriomeningitis Virus (LCMV) in Birmingham, Alabama"

1991 MPH
Department of Epidemiology and International Public Health.
School of Public Health, University of Alabama at Birmingham. Birmingham, AL.

1987 MS
Department of Microbiology,
School of Natural Science, University of South Florida. Tampa, FL
"Exoantigen comparisons of selected isolates of Basidiobolus species."

1982 BS
Department of Biology.
Yonsei University. Seoul, S. Korea.

Work Experience

Aug. 2001-present
Assistant Professor
H. Lee Moffitt Cancer Center,
Department of Interdisciplinary Oncology
School of Medicine, University of South Florida, Tampa, FL

Aug. 1999-Aug. 2001
Research Assistant Professor
H. Lee Moffitt Cancer Center,
Department of Interdisciplinary Oncology
School of Medicine, University of South Florida, Tampa, FL

Jan. 1996-Jul. 1999
Post-doctoral Fellow
Dept. of Pathology and Laboratory Medicine,
School of Medicine, Temple University, Philadelphia, PA

Jan. 1990-Aug. 1992
Research Assistant II
Dept. of Comparative Medicine,
School of Medicine, University of Alabama at Birmingham, Birmingham, AL

Mar. 1988-Jan. 1990
Associate Microbiologist

Div. of Biochemistry
Southern Research Institute, Birmingham, AL

Nov. 1985-Mar. 1988
Research Assistant.
Div. of Infectious and Tropical Diseases
James A. Haley Veterans Hospital, Tampa, FL

Dec. 1981-Dec. 1982
Biologist
Div. of Research and Development, Central Research Institute.
Green Cross Pharmaceutical Company, Seoul, S. Korea.

Ad Hoc reviewer

1. European Journal of Cancer (1999)
2. External Reviewer, The Department of Veterans Affairs (2001)
3. Internal Reviewer, The Advanced Cancer Detection Center, H. Lee Moffitt Cancer Center (2001)
4. External Reviewer, The Department of Veterans Affairs (2002)

Symposia

1 Co-Chair of Epidemiology II, scientific section, 5th research workshop on the Biology, Prevention and Treatment of Head and Neck Cancer, Mclean VA. Aug. 1998.

Membership

Associated member, American Society of Microbiology (1983-1988)
Associated member, American Society of Virology (1989-1995)
Member in Residence, H Lee Moffitt cancer center and Research Institute (1999-present)
Member, American Association for Cancer Research (#14203) (1996- present)
Molecular Epidemiology Group (2000-present)
Associate member, Early Detection Research Network (EDRN)/NCI (2000-present)

Special Award

1. Outstanding presentation award: The Korean Scientists and Engineers Association in America. Florida Chapter. The 9th annual Mini-Symposium. Dec. 15, 1984, Gainesville, FL.

2. Travel award: The 3rd annual meeting of Florida Society of Electron Microscopy. March 13-15,1985. Tampa, FL.

3. Pre-doctoral fellowship for U.S. Public Health Service Trainee: Jan. 1990-Dec. 1994, School of Public Health, University of Alabama at Birmingham.

4. Travel award: The 13th Annual Meeting-American Society for Virology. July 9-13, 1994. Madison, WI

5. Young Investigator award: The 89th Annual Meeting American Association for Cancer Research. March 28- April 1, 1998. New Orleans, LA.

6. Outstanding Abstract award: abstract was presented in the late breaking session (1988). The 89th Annual Meeting American Association for Cancer Research. March 28- April 1, 1998. New Orleans, LA.

7. Travel award: The 5th Research workshop on the biology, prevention and treatment of head and neck cancer. August 26- 30, 1998. McLean, VA.

8. Outstanding abstract award: selected as one of the best three abstracts. The 5th Research workshop on the biology, prevention and treatment of head and neck cancer. August 26-30, 1998. McLean, VA

9. NIH/NCI Individual National Research Service Awards (F32CA73173) (PI: Jong Park)
NIH/NCI/ Div. of Cancer treatment, diagnosis and centers. 09/01/97-08/30/00
$34,000/yr, 3 years
Title: Xenobiotic-metabolizing enzymes and oral cancer susceptibility

10. University South Florida/Institute of Black Life/ Faculty Grant award (PI: Jong Park), "Examination of role of epoxide hydrolase genetic polymorphism in tobacco-related cancer risk in African Americans." Period: March 2000-March 2001. $1,500

11. American Cancer Society/IRG: (PI: Jong Park), "Analysis of epoxide hydrolase polymorphism." Period: June 2000-June 2001. $20,000

12. DAMD17-01-2-0056 (PI: Jong Park) 10/01/01-9/30/03
DOD/Advance Cancer Detection Center $92,500/yr, 2 years
"Epoxide hydrolase and its functional significance."

Active Award

U01CA084973-01 (PI. David Sidransky) 05/15/01-5/14/04 no-cost extension (until April 23, 2004) NCI/Early Detection Research Network $93,932/yr, 1 year 30%
Epoxide hydrolase polymorphism and lung cancer.
The major goal of this project is to investigate prevalence of genetic polymorphisms of epoxide hydrolase in lung cancer patients.

1R03CA91314-01 (PI: Jong Park) 06/01/01-05/31/03 no-cost extension (until May 30, 2004)
NIH/NCI $50,000/yr, 2 years 30%
Elucidation of epoxide hydrolase polymorphisms.
The major goal of this project is to investigate the association between genotypes epoxide hydrolase and phenotypes.

Pending

R21CA106776 (PI:Jong Park) 05/01/2004-4/30/2006
NIH/NCI R21 $100,000/yr, 2 yr

"DNA Repair Enzymes and Prostate Cancer Risk"
Submitted June 2002
The major goal of this project is to investigate the association between genotypes of genes involve DNA repair process and risk for prostate cancer.

K22 CA098579 (PI: Jong Park), 03/01/2004-2/28/2006
NIH/NCI K22 $135,662/yr, 3 yrs
Submitted Nov. 2002 scored 227, resubmitted July 2003
"Genetic Polymorphisms and Risk for Prostate Cancer"
The major goal of this project is to investigate the association between genotypes of genes involve steroid hormone metabolism and risk for prostate cancer.

Publications:

1. Yanco, B. G., Nettlow, A., Okafor, J. I., **Park, J.**, and TeStrake, D. (1986) Comparative antigenic studies of species of Basidiobolus and other medically important fungi. J. of Clin. Microbiol. 23(4):679-682.

2. Ganguly, R., and **Park, J**. (1988) Immunostimulating agent against Influenza virus infection in senescent rats. Allerg. Immunol. 34:239-247.

3. Testrake, D., **Park, J.**, and Yanco, B. G. (1989) Exoantigen comparisons of selected isolates of Basidiobolus species. Mycologia 81(2):284-288.

4. Wille, J.J., **Park, J**., and Elgavish, A. (1992) Effects of growth factors, hormones, bacterial lipopolysaccharide, and lipotechoic acids on the clonal growth of normal ureteral epithelial cells in serum-free culture. J. of Cell. Physiol. 150:52-58

5. Stephensen, C. B., Blount, S. R., Schoeb, T. R. and **Park, J**. (1993) Vitamin A deficiency impairs some aspects of host response to influenza A virus infection in BALB/c mice. J. Nutr. 123:823-833.

6. Stephensen, C. B., **Park, J.**, and Blount, S. R. (1995) Genomic sequence analysis confirms that the etiologic agent of Callitrichid Hepatitis is LCMV. J. Virol. 69:1349-1352.

7. **Park, J.**, Peters, C. J., Rollin, P, E., Ksiazek, T. G., Gray, G., Waites, K. B., and Stephensen, C. B (1997). Development of a reverse transcription-polymerase chain reaction assay for diagnosis of lymphocytic choriomeningitis virus (LCMV) infection and its use in a prospective surveillance study. J. Med. Virol. 51:107-114

8. **Park, J.**, Peters, C. J., Rollin, P. E., Ksiazek, T. G., Katholi, C. R., Waites, K. B., Gray, B., Maetz, H. M., and Stephensen, C. B.(1997). Age distribution of lymphocytic choriomeningitis virus serum antibody in Birmingham, Alabama: Evidence of Decreased risk of Infection. Am. J. Trop. Med. Hyg. 57(1):37-41.

9. **Park, J.**, Muscat, J.E., Ren, Q., Schantz, S. P., Harwick, R. D., Stern, J. C., Pike, V., Richie, J. P. Jr., and Lazarus, P. (1997). CYP1A1 and GSTM1 polymorphisms and oral cancer risk. Cancer Epi. Biom. Prev. 6:791-797.

10. Lazarus, P., Sheikh, S.N., Ren, Q., Schantz, S. P., Stern, J. C., Richie, J. P. Jr., and **Park, J.** (1998) p53 but not p16 mutations in oral squamous cell carcinomas are associated with specific CYP1A1 and GSTM1 polymorphic genotypes and patient tobacco use. Carcinogenesis 19(3):509-514.

11. Qin, G-Z., **Park, J.Y.**, Chen S-Y. and Lazarus, P. (1999) A high prevalence of p53 mutations in premalignant oral erythroplakia. Int. J. Cancer 80:345-348.

12. **Park, J.Y.,** Schantz, S.P. Stern, J.C., Kaur, T., and Lazarus, P. (1999) Association between GSTPi genetic polymorphisms and oral cancer risk. Phamacogenetics 9:497-504

13. Ren, Q., Murphy, S. E., Dannenberg, A., **Park, J.Y.,** Tephly, T.R., and Lazarus, P. (1999) Glucuronidation of the lung carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol by rat UDT-glucuronosyltransferase 2B1. Drug metabolism and Disposition 27:1010-1016.

14. **Park, J.Y**., Muscat, J.E., Schantz, S.P. Stern, J.C., Kaur, T., Richie, J.P., and Lazarus, P. (2000) Comparison of GSTM polymorphisms and risk for oral cancer between African Americans and Caucasians. Pharmacogenetics 10:1-9.

15. Lazarus, P and **Park, J. Y**. (2000) metabolizing enzyme genotype and risk for upper aerodigestive tract cancer. (review), Oral Oncology 36:421-431.

16. Liu, S., **Park, J.Y.** Schantz, S.P. Stern, J.C., and Lazarus, P., (2001) Association of CYP2E1 Rsa1/Pst1 polymorphism with oral cancer risk. Oral Oncology 37(5);437-445.

17. Zheng, Z., **Park, J.**, Guillemette, C., and Lazarus, P (2001) The tobacco carcinogen-detoxifying enzyme UGT1A7 and its association with orolaryngeal cancer risk. Journal of National Cancer Institute 93 (18):1411-18.

18. Elahi, A., Zhong, Z, **Park, J.,** Eyring, K., McCaffrey T., and Lazarus, P (2002) The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. Carcinogenesis 23:1229-1234.

19. **Park, J.,** Schantz SP and Lazarus, P. (2003) Epoxide hydrolase genotype and orolaryngeal cancer risk: Interaction with GSTM1 genotype. Oral Oncology 39:(5) 483-490.

20. Chen, L, Elahi, A., Pow-Sang, J., Lazarus, P, and **Park, J** (2003) Association between polymorphism of human oxoguanine glycosylase 1 (hOGG1) and risk for prostate cancer. (in press, The Journal of Urology, Dec)

21. Kim, JS, Choi, MA, Chung, WY, **Park, JY**, Cho, KS, and Park, KK (2004).Effects of polymorphisms of xenobiotic metabolizing enzymes and their roles in risk for periodontal disease. (Accepted Journal of Clin. Periodontal. 2004)

22. Chen, L., Tockman M, Elahi, A., Lazarus, P., and **Park. J.** (2004). Genetic analysis of microsomal epoxide hydrolase gene and its association with lung cancer risk. (Accepted International Journal of Cancer. 2004)

23. Chen L., Shade, K, Lazarus, P, Seigne, J, Patterson, S., Helal, M, Pow-Sang, J, and **Park, J.** (2004) Asp326Tyr polymorphism in UDP-glucuronosyltransferase (UGT) 2B15 gene and risk of prostate cancer among Caucasians (submitted to the Journal of Urology)

24, **Park. J.** Chen, L., Tockman M, Elahi, A., and Lazarus, P. (2004). The human OGG1 DNA repair enzyme and its association with lung cancer risk. (Submitted to Phamakogenetics)

25. Muscat J, Kim W, Collisimo S, Mellikian A, Lazarus P, **Park JY**, Stellman, SD and Richie, JP Jr. (2004). Effects of cigarette smoking, smoking biomarkers, and GSTM1 and GSTM3 genetic polymorphisms on blood glutathione concentrations. (submitted).

26. Cha, IH, Choi, MA, **Park, JY**, Chung, WY, and Park, KK (2004). Polymorphism of the *CYP1A1* and *GSTM1* gene and susceptibility to oral cancer in a Korean population (Submitted Cancer Letter)

27. Park, J. Chen, L, and M. Tockman. (2004) Microsomal epoxide hydrolase genotype and its association with risk for COPD (Preparation).

28, Park, J. Chen, L and M. Tockman. (2004) Genetic polymorphisms of neutrophil elastase promoter region and risk for lung cancer (Preparation).

Abstracts:

1. Kim, K., Oh, S., Park, Y., Park, S., Ahn, C., and **Park, J.** (1982) Biochemical studies for interferon from human leukocytes. The Korean Journal of Virology 12:90. The 14th Annual meeting of Korean Society for Virology. Nov. 27. Shingal, S. Korea.

2. Nettlow, A., **Park, J.**, Yanco, B., and TeStrake, D. (1984) Preliminary exoantigenic studies of several species of Basidiobolus. Abstracts. p6. Southeastern branch of American Society for Microbiology. Oct. 25-27. Clearwater, FL. (oral presentation)

3. Ledford, D. K., Lockey, R. F., Serbousek, D., **Park, J.**, and Testrake, D, (1984) Studies on airborne pollens and fungi during grass mowing. Abstracts, p6. Southeastern Branch of American Society for Microbiology. Oct, 25-27. Clearwater, FL.

4. **Park, J.** (1984) Identifying Basidiobolus employing the use of exoantigens. The Korean scientists and engineers association in America. News letter. Florida Chapter. p10. The 9th annual mini-symposium. Dec. 15. Gainesville, FL. (oral presentation)

5. **Park, J.**, Brown, J., Yanco, B., and TeStrake, D. (1985) Ultrastructural studies of an isolates of Basidiobolus. Proceedings of the third annual meeting. p19. The 3rd annual meeting of Florida society of electron microscopy. March 13-15, Tampa, FL. (oral presentation)

6.Yanco, B. G., Nettlow, A., **Park, J.**, and TeStrake, D. (1985) Antigenic comparison of Basidiobolus. Abstracts of Annual meeting of American Society for Microbiology, p373, March. 1985. Las Vegas, NV.

7. **Park, J.**, Nettlow, A., Yanco, B. G., and TeStrake, D. (1985) Electrophoretic characterization of exoantigens of four isolates of Basidiolobus. Southeastern and South Carolina

branches, American Society for Microbiology. Proceedings of annual meeting, p6, October 17-19. Savannah, GA. (oral presentation)

8. Yanco, B. G., Nettlow, A., Okafor, J. I., **Park, J.**, and Testrake, D. (1985) Antigenic relationships among species of Basidiobolus and Conidiobolus as determined by an immunodiffusion test exoantigen comparison of Basidiobolus. Southeastern and South Carolina branches, American Society for Microbiology. Proceedings of annual meeting, p6, October 17-19. Savannah, GA.

9. Yanco, B., Nettlow, A., **Park, J.**, Okafor, J., Testrake, D., and Lackman, C. S. (1986) Antigenic relationship among species of Basidiobolus as determined by crossed immunoelectrophoresis. Abstracts of Annual Meeting, American Society for Microbiology. March 23-28. Washington, D.C.

10. Nettlow, A. L., Brown, J. K., **Park, J.**, Wain, R. P., Yanco, B. G., Okafor, J., and Testrake, D. (1986) Immunologic and electrophoretic studies of species of Basidiobolus. The 9th annual meeting international society for human and animal mycology, May, 19-24. Atlanta, GA.

11. Ganguly, R., and **Park, J**. (1987) Age effects on lung immune responses to intranasal influenza subunit vaccine. Abstracts of annual meeting of American Society for Microbiology, p25. March, 1987.

12. Stephensen, C. B., Blount, S. R., **Park, J.** and Schoeb, T. R. (1992) Virus clearance and mortality are not affected by vitamin A deficiency during experimental influenza A virus infection of Balb/c mice. FASEB J. 6:A1662. Anaheim, CA.

13. **Park, J.**, Stephensen, C. B., and Peters, C. J. (1994) Development of PCR assay for Lymphocytic Choriomeningitis virus (LCMV). The 3rd Regional virology conference. Georgia State University. March 18-20. Atlanta, GA. (oral presentation)

14. **Park, J.**, Stephensen, C. B., Ksiazek, T. G., Waites, K, B., and Peters, C. J. (1994) Development and application of a PCR assay for Lymphocytic Choriomeningitis Virus (LCMV). Scientific Program and Abstracts. p172. The 13th annual meeting-American Society for Virology. University of Wisconsin-Madison, July 9-13. Madison, WI. (oral presentation)

15 **Park, J.**, Ren, Q., Strudwick, S., Muscat, J., Schantz, S., Harwick, R., and Lazarus, P (1996) GSTM1 and CYP1A1 polymorphisms and oral cavity cancer risk. Proceedings of the American Association for Cancer Research. 37:107, The 87th Annual meeting American Association for Cancer Research. April 20-24. Washington, DC.

16. **Park, J.**, Sheikh, S., Schantz, S., Stern, J., Garewal, H., Sciubba, J., Muscat, J., Richie, J. P., Ren, Q., and Lazarus, P. (1996) Molecular markers of oral cancer susceptibility. The Second International Meeting of Genetic Epidemiology, June 26-28, Baltimore, MD, Proceedings of Genetic Epidemiology of Cancer.

17. **Park, J.**, Kaur, T., Schantz, S., Harwick, R., Stern, J. Muscat, J. and Lazarus, P. (1997) Interethnic variability and genetic linkage in GSTM1 and GSTM3 polymorphic frequencies. Proceedings of the American Association for Cancer Research. 38:213,

The 88th Annual Meeting American Association for Cancer Research. April, 12-16. San Diego. CA.

18. **Park, J.**, Ren, Q., Schantz, S., Stern, J., Richie, J. P., Sheikh, S., and Lazarus, P. (1998) p53 mutations in oral tumors are associated with specific CYP1A1 and GSTM1 polymorphic genotypes and patient tobacco use. Proceedings of the American Association for Cancer Research. 39:183, The 89th Annual Meeting American Association for Cancer Research. March 28- April 1. New Orleans, LA.

19. Ren, Q., **Park, J.**, Dannenberg, A..J. Yang, E.K., Murphy, S.E., Tephly, T., and Lazarus, P. (1998) Glucuronidation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) by rat UDP-glucuronsyltransferases (UGT). Proceedings of the American Association for Cancer Research. 39:330, The 89th Annual Meeting American Association for Cancer Research. March 28- April 1, 1998. New Orleans, LA..

20. Qin, G-Z., **Park, J.**, Pundru, A., Chen, S-Y, and Lazarus, P. (1998) p53 mutations in oral premalignant lesions: Correlation with malignant potential. Proceedings of the American Association for Cancer Research. 39:476, The 89th Annual Meeting American Association for Cancer Research. March 28- April 1, 1998. New Orleans, LA.

21 Liu, J., **Park, J.**, Schantz, S., Kaur, T., Stern, J., and Lazarus, P. (1998) Influence of smoking and alcohol consumption on genotype-associated susceptibility to oral cancer. Proceedings of the American Association for Cancer Research. Vol39:627, The 89th Annual Meeting American Association for Cancer Research. March 28- April 1, 1998. New Orleans, LA.

22. **Park, J.** Muscat, J.E., Schantz, S.P. Stern, J.C., Richie, J.P., and Lazarus, P., (1998) Association between GSTM polymorphisms and risk for oral cancer in African Americans as compared to Caucasians. The 89th Annual Meeting American Association for Cancer Research. March 28- April 1, 1998. New Orleans, LA. (presented in late breaking session)

23. **Park, J**. Muscat, J.E., Schantz, S.P. Stern, J.C., Richie, J.P., and Lazarus, P., (1998) GSTM genotype and risk for oral cancer in African Americans and Caucasians. Head & Neck 20(5):470. The 5th Research workshop on the biology, prevention and treatment of head and neck cancer. August 26- 30, 1998. McLean, VA. (oral presentation)

24. **Park, J.**, Liu, S.P. Schantz, S., Stern, J., and Lazarus, P. (1998) Oral cancer risk associated with polymorphisms in genes coding for the GST Pi and CYP2E1 enzymes. Head & Neck 20(5):470. The 5th Research workshop on the biology, prevention and treatment of head and neck cancer. August 26- 30, 1998. McLean, VA

25. **Park, J.**, Leon, J., Stern. JC. Schantz sp, Alvi, A and Lazarus, P (1999) Role of the CYP1B1 polymorphism and oral cancer risk. Proc. Amer. Assoc. cancer res. 40:566, 1999. The 90th Annual Meeting American Association for Cancer Research. April 1-5, 1999. Philadelphia, PA.

26. **Park, J.,** Liu, J., Stern. JC. Schantz SP, Alvi, A and Lazarus, P (1999) Association between GSTPi polymorphisms and oral cancer risk . Proc. Amer. Assoc. cancer res. 40:566,

1999. The 90th Annual Meeting American Association for Cancer Research. April 1-5, 1999. Philadelphia, PA.

27. **Park, J.,** and Lazarus, P (2001) Epoxide hydrolase polymorphism and oral cancer risk: correlation with the GSTM1-null genotype. Proc. Amer. Assoc. cancer res. 40:566, 2001. The 92nd Annual Meeting American Association for Cancer Research. March 24-28, 2001. New Orleans, LA.

28. Zheng, Z., **Park, J.,** Guillemette, C., and Lazarus, P (2001) The tobacco carcinogen-detoxifying enzyme UGT1A7: expression in aerodigestive tract tissues and importance of UGT1A7 alleles in risk for oral cancer. Proc. Amer. Assoc. cancer res. 40:566, 2001.. The 92nd Annual Meeting American Association for Cancer Research. March 24-28, 2001. New Orleans, LA.

29. Elahi, A., **Park, J.,** and Lazarus, P (2001) Screening of P21 genetic polymorphisms and the association between the P21 codon 31 (ser/arg) polymorphism and risk for lung cancer. Proc. Amer. Assoc. cancer res. 40:566, 2001. The 92nd Annual Meeting American Association for Cancer Research. March 24-28, 2001. New Orleans, LA.

30. Chen, L., Tockman, M. and **Park, J.** (2002) Epoxide hydrolase polymorphisms and risk for lung cancer. NIH/NCI/Early detection research network (EDRN). The 5th Steering Committee Meeting. February 3-5, 2002. M. D. Anderson Cancer Center, Houston, TX

31. Chen, L., Lazarus, P., Tockman, M. and **Park, J.** (2002) Epoxide hydrolase polymorphisms and risk for lung and oral cancer. Gordon Research conferences. New Frontiers in Cancer Detection & Diagnosis. March 10-15, 2002. Ventura, CA

32. Elahi, A., Zhong, Z., Eyring K., **Park, J.,** Hall K., Muffly, B., Mericle L., Griffin S., Wiener D. and Lazarus, P (2002) Role of the ser326 polymorphism in the hOGG1 DNA repair enzyme in risk for orolaryngeal cancer. The 93rd Annual Meeting American Association for Cancer Research. April 6-10, 2002. San Francisco, CA.

33. Chen L., Dossett N., Pow-Sang J., Lazarus P., and **Park J**. (2002) Human UDP glucuronosyltransferase (UGT) 2B15 polymorphism and prostate cancer risk. The 93rd Annual Meeting American Association for Cancer Research. April 6-10, 2002. San Francisco, CA.

34. Wille J. J., and **Park, J.** (2002) c-Neu and phosphotyrosine expression in proliferation keratinocyte cultures exposed to retinoic acid and tetradecanoyl phorbol acetate. The Society for Investigative Dematology May 16th 2002.

35. Muscat J, Kim W, Collisimo S, Mellikian A, Lazarus P, **Park JY**, Stellman, SD and Richie, JP Jr. (2002). Effects of cigarette smoking, smoking biomarkers, and GSTM1 and GSTM3 genetic polymorphisms on blood glutathione concentrations. Frontiers in Cancer Prevention Research: October 14-18, 2002. Boston, MA

36. Chen L., Elahi, A., Pow-Sang, J., Lazarus, P., Eyring, K., and **Park J**. (2003). The human oxoguanine glycosylase 1 (hOGG1) DNA repair enzyme and its association with prostate cancer risk. The 94th Annual Meeting American Association for Cancer Research. July 11-14, 2003. Washington DC.

37. Elahi, A., Bendaly, J., **Park J.**, Wiener, D. and Lazarus, P. (2003). African Americans with the UGT1A10 glu139lys polymorphism exhibit significantly lower risk for orolaryngeal cancer. The 94th Annual Meeting American Association for Cancer Research. July 11-14, 2003. Washington DC.

38. **Park, J.**, Chen, L., Choksi, N. Tockman, M., Elahi, A. and Lazarus, P. (2003). The human oxoguanine glycosylase 1 (hOGG1) DNA repair enzyme and its association with lung cancer risk. SNPs, Haplotypes, and Cancer: Applications in Molecular Epidemiology, AACR Conference, September 13-17, 2003, Key Biscayne, FL.

Lectures, Poster presentations, Workshops, and Conference attended

1. The 14th Annual meeting of Korean Society for Virology. November 27, 1982. Shingal, S. Korea.

2. The 1st Annual meeting of Florida Society for Electron Microscopy. March, 1983. Tampa, FL.

3. The Annual meeting of Southeastern branch, American Society for Microbiology. October 25-27, 1984. Clearwater, FL. (Oral presentation)

4. The 9th Annual mini-symposium, The Korean Scientists and Engineers Association in America. Florida Chapter. December 15, 1984. Gainesville, FL. (Oral presentation)

5. The 3rd Annual meeting of Florida Society for Electron Microscopy. March 13-15, 1985. Clearwater, FL. (Oral presentation)

6. The Annual meeting of American Society for Microbiology. March 1985. Las Vegas, NV.

7. The Annual meeting of Southeastern and South Carolina Branches, American Society for Microbiology. October 17-19, 1985. Savannah, GA. (Oral presentation)

8. The 3rd Southeastern Regional Virology Conference. Georgia State University, March 18-20. 1994. Atlanta, GA. (Oral presentation)

9. The 13th Annual meeting of American Society for Virology. University of Wisconsin-Madison. July 9-13, 1994. Madison, WI. (Oral presentation)

10. The 87th Annual meeting of American Association for Cancer Research. April 20-24, 1996. Washington, DC.

11. The 88th Annual meeting of American Association for Cancer Research. April 12-16, 1997. San Diego, CA.

12. The 89th Annual meeting of American Association for Cancer Research. March 28-April 1, 1998. New Orleans, LA.

13. The 5th Research workshop on the Biology, Prevention and Treatment of Head and Neck Cancer. August 26-30, 1998. McLean, VA. (Oral presentation)

14. The 90th Annual meeting of American Association for Cancer Research. April 1-5, 1999. Philadelphia, PA.

15. The 91st Annual meeting of American Association for Cancer Research. April 1-5, 2000. San Francisco, CA.

16. NIH/NCI/Early detection research network (EDRN). The 1st Annual Scientific Workshop. September 26-27, 2000. Northwestern Memorial Hospital, Northwestern University, Chicago, IL.

17. Advanced Cancer Detection Center/DOD external advisory meeting, Nov. 13 2000 (Oral presentation), H. Lee Moffitt Cancer Center. Tampa, FL

18. The 92nd Annual meeting of American Association for Cancer Research. March 24-28, 2001. New Orleans, LA

19. NIH/NCI/Early detection research network (EDRN). The 2nd Annual scientific workshop. October 14-16, 2001. Seattle, WA.

20. NIH/NCI/Early detection research network (EDRN). The 5th Steering Committee Meeting. February 3-5, 2002. M. D. Anderson Cancer Center, Houston, TX. (Oral presentation)

21. Gordon Research Conferences. New Frontiers in Cancer Detection & Diagnosis. March 10-15, 2002. Ventura, CA

22. The 93rd Annual meeting of American Association for Cancer Research. April 6-10, 2002. San Francisco, CA.

23. Interdisciplinary Center for Biotechnology Research. "Applications in Apoptosis, Cell Viability and Signaling: Making Sense of Your Options Workshop" February 18-19, 2003. Gainesville, FL.

24. NIH/NCI/Early detection research network (EDRN). The 8th Steering Committee Meeting. June 23-25, 2003. Ronald B. Herberman Conference Center, UPMC Cancer Pavilion. University of Pittsburgh, Cancer Institute, Hillman Cancer Center. Pittsburgh, PA.

25. SNPs, Haplotypes, and Cancer: Applications in Molecular Epidemiology, AACR meeting September 13-17, 2003, Key Biscayne, FL.

Presentation

Oct. 25 1984. Oral presentation. "Basidiobolus exoantigen" The Annual meeting of Southeastern Branch, American Society for Microbiology. October 25-27, 1984. Clearwater, FL.

Dec.15 1984. Oral presentation. "comparison of Basidiobolus exoantigen" The 9th Annual mini-symposium, The Korean Scientists and Engineers Association in America. Florida Chapter. December 15, 1984. Gainesville, FL.

Mar. 14.1985. Oral presentation, "Characters of Basidiobolus" The 3rd Annual meeting of Florida Society for Electron Microscopy. March 13-15, 1985. Clearwater, FL.

Oct. 17. 1985. Oral presentation, "Imunodetection of Basidiobolus" The Annual meeting of Southeastern and South Carolina Branches, American Society for Microbiology. October 17-19, 1985. Savannah, GA.

Mar. 19 1994. Oral presentation, " PCR detection of Lymphocytic choriomeningitis visus" The 3rd Southeastern Regional Virology Conference. Georgia State University, March 18-20. 1994. Atlanta, GA.

Jul. 12 1994. Oral presentation, "screening of LCMV" The 13th Annual meeting of American Society for Virology. University of Wisconsin-Madison. July 9-13, 1994. Madison, WI.

Aug. 27, 1998. Oral presentation. "cancer risk" The 5th Research workshop on the Biology, Prevention and Treatment of Head and Neck Cancer. August 26-30, 1998. McLean, VA.

Nov. 13, 2000. Oral presentation. "EH polymorphism and cancer risk" Advanced Cancer Detection Center/DOD external advisory meeting, Nov. 13 2000, H. Lee Moffitt Cancer Center. Tampa, FL

Dec. 8, 2000. Oral presentation. "Genetic polymorphism and smoking related cancer risk" Research in progress. Dec. 8, 2000 H. Lee Moffitt Cancer Center Tampa, FL

Feb. 5, 2002. Oral presentation. "Genetic polymorphism of EH and lung cancer risk" NIH/NCI/Early Detection Research Network (EDRN). The 5th Steering Committee Meeting. February 3-5, 2002. M. D. Anderson Cancer Center, Houston, TX

Feb. 11, 2002. Oral presentation. "UGT2B15 polymorphims and prostate cancer risk" GU weekly clinical conference. H. Lee Moffitt Cancer Center, Tampa. FL

Jun. 14, 2002. Oral presentation. Grand Round, Korean National Cancer Center "Genetic polymorphisms of tobacco carcinogen metabolizing enzymes and individual cancer risk" Seoul, S. Korea

Jun. 15, 2002. Oral presentation. Departmental seminar, School of Medicine, Department of Preventive Medicine. Seoul National University "Importance of UDP-glucuronosyl transferases (UGT) in Susceptibility to Cancers". Seoul, S. Korea

Jun. 17, 2002 Oral presentation Departmental Seminar, School of Natural Science. Department of Biology. Yonsei University. "Genetic polymorphisms of metabolizing enzymes and individual cancer risk cancer risk" Seoul, S. Korea

July 21, 2003. Oral presentation. Press Conference "The human oxoguanine glycosylase 1 (hOGG1) DNA repair enzyme and its association with prostate cancer risk". The 94th Annual Meeting American Association for Cancer Research. Washington DC.

Genetic analysis of microsomal epoxide hydrolase gene and its association with lung cancer risk.

Lan Chen[1], Melvyn Tockman[2], Abul Elahi[1], Philip Lazarus[1], and Jong Y. Park[1*]

[a]Department of Interdisciplinary Oncology, [b]Molecular Screening Program, 12902 Magnolia drive, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612. USA

*Corresponding author. Fax: (813) 632-1720;

Address: Department of Interdisciplinary Oncology, 12902 Magnolia drive, OSWFBB, H. Lee Moffitt Cancer Center and Research Institute, Tampa, FL 33612 USA

E-mail: parkj@moffitt.usf.edu.

Abstract

The human microsomal epoxide hydrolase (EH) gene contains polymorphic alleles, which may be linked to increased risk for tobacco-related lung cancer. The purpose of this study was to screen new polymorphisms and determine whether these polymorphisms can be used to predict individual susceptibility to lung cancer. The PCR-single strand conformation polymorphism (SSCP) analysis was used to screen for polymorphisms in the coding region of the EH gene. Eleven polymorphisms, including previously reported polymorphisms were identified and the prevalence of these variants was assessed in at least 50 healthy Caucasians and African Americans, respectively. Among the 11 polymorphisms, the prevalence of the codons 43, 113 and 139 amino acid changing EH polymorphisms were examined in 169 Caucasian incident cases with primary lung cancer, as well as in individually-matched controls to examine the role of EH polymorphisms in lung cancer risk. Significantly increased risk for lung cancer was observed for subjects with both the heterozygous EH $113^{His}/113^{Tyr}$ genotype (OR = 2.8, 95% CI = 1.3 – 6.1) and the homozygous EH $113^{Tyr}/113^{Tyr}$ genotype (OR = 2.4, 95% CI = 1.1 – 5.0). A significant increase in risk for lung cancer was observed for both predicted intermediate (OR = 2.7, 95% CI=1.3-5.9) and high EH activity genotypes (OR=5.0, 95% CI=1.9-13.3) as compared to low EH activity genotypes . A significant association was found between predicted EH activity genotypes and lung cancer risk with a dose–effect relationship (trend test: $p<0.02$). These results suggest that EH polymorphisms may play significant roles in risk for lung cancer.

Key Words: Epoxide hydrolase, lung cancer, genetic polymorphism, molecular epidemiology, Individual cancer susceptibility.

Introduction

Genetic polymorphisms are associated with a number of genes that code for enzymes involved in the metabolic activation or detoxification of carcinogens. Large differences have been described among individuals for activities of several carcinogen metabolizing enzymes and it has been shown that enzyme activity-altering polymorphisms may influence individual cancer risk [1-5].

Epoxide hydrolase (EH) enzyme cleaves a range of alkene and arene oxides to form *trans*-dihydrodiols. For some polycyclic aromatic hydrocarbons (PAHs) including benzo(a)pyrene (BaP), the dihydrodiol derivatives are substrates for additional metabolism to more highly reactive and carcinogenic compounds. In two previous studies of a small number of subjects who had adverse reaction to anticonvulsant drugs [6, 7], several EH variants were identified but none were associated with risk for hypersensitive reactions. Other studies have shown that two amino acid-altering polymorphisms located at codons 113 and 139 are associated with alterations in EH activity. The EH^{113His} variant is associated with a 40% decrease in EH activity while the EH^{139Arg} variant enhances enzyme activity by 25% via an increase in EH protein stability [8]. These polymorphic alleles have previously been linked to risk change for colon [9], ovarian [10], and tobacco-related cancers, such as lung[4, 11-16] and oral cavity[17]. Together with the finding that EH is expressed in lung tissue [18], these data suggest that EH polymorphisms may play a role in risk for lung cancer. The goals of the present study were to screen and identify EH polymorphisms in multiple racial groups (ie, African Americans and Caucasians) and determine whether they may be associated with increased risk for lung cancer.

Materials and Methods

Study Population

The subjects screened in this study were recruited as part of on-going studies examining genetic risk factors for tobacco-related cancers [5, 19]. For the identification of EH polymorphisms (PCR-SSCP) in different racial groups, control subjects from New York City area were included 40 Caucasians and African Americans, respectively. To assess the prevalence of polymorphisms found in SSCP analysis, subjects from same area were included at least 50 Caucasians and 50 African Americans. All subjects for screening polymorphisms were recruited after an initial verbal screening to determine that they had no previous diagnosis of cancer.

For the investigation roles of EH polymorphisms in risk for lung cancer, all subjects were recruited from H. Lee Moffitt Cancer Center (Tampa, FL). All cases were patients diagnosed with primary lung cancer and were identified between 1999 and 2002. All cases were diagnosed within one year prior to recruitment into the study, and were histologically confirmed by the Pathology Department at the H. Lee Moffitt Cancer Center. Ninety-five percent of case subjects who were asked to participate in the study consented.

We recruited potential control subjects at the Lifetime Cancer Screening Center affiliated at the H. Lee Moffitt Cancer Center. At this center, which screens approximately 22,000 subjects annually, routine screenings are performed for cancers of the breast, prostate, colorectum, cervix and skin. All control subjects were recruited

after an initial verbal screening to determine that they had no previous diagnosis of cancer, and none of the controls recruited into this study were diagnosed with any form of cancer or premalignancy after screening. The eligible pool of control subjects was restricted to those individuals with the same age at diagnosis (± 5 years), race, and sex as the case subjects, with controls matched in a 1 to 1 ratio with cases. Eighty-three percent of the control subjects who were asked to participate in the study consented. For cases, buccal cell (n=126) or blood samples (n=43), collected at a follow-up examination were used for the analysis of polymorphic genotypes, while blood samples were collected for the analysis in controls (n=169). Protocols involving the analysis of buccal cell and blood specimens were approved by the institutional review board at the H. Lee Moffitt Cancer Center and informed consents were obtained from all subjects. A questionnaire that contained questions on demographics and life-long smoking habits was administered to all study subjects. Tobacco use was categorized into pack-years (py) for smokers of cigarettes (1 py equaled one pack of cigarettes per day for 1 year), cigars (1 py equaled four cigars per day for 1 year), and pipe tobacco (1 py equaled five pipes per day for 1 year) according to the criteria described by Benhamou et al[20]. Study subjects who smoked 100 or fewer cigarettes in their lifetime (the equivalent of 0.014 or fewer py) were categorized as never-smokers.

PCR-SSCP and PCR-RFLP analysis of the EH gene

Genomic DNA was isolated from oral buccal cells or blood from non-cancer controls by incubation overnight with proteinase K (0.1 mg/ml) in 1% sodium dodecyl sulfate at

50°C and extracted with phenol:chloroform, and ethanol precipitation as previously described [21].

We utilized PCR-SSCP analysis to identify genetic polymorphisms in the EH gene. EH intron-specific sense and antisense primers homologous to intron sequences immediately adjacent to EH coding exons and splice sites were used for all SSCP analysis. The primer sequences, annealing temperatures for PCR and expected PCR fragment sizes are outlined in Table 1. Screening for EH polymorphisms was performed by PCR amplification of genomic DNA from both African Americans (n=40) and Caucasians (n=40), and SSCP analysis of amplified exons. Both Caucasians and African Americans were screened to eliminate the possibility that race-specific polymorphisms would be missed in this analysis. SSCP analysis of radio-labeled PCR products was performed as previously described [22]. When results of SSCP analysis were suggested potential polymorphisms by shifted SSCP band patterns, the exact nature of the polymorphic EH sequence were elucidated directly from the shifted band on the SSCP gel by standard dideoxy sequencing [23]. All DNA sequencing was performed at the Molecular Biology Core Facility located in the H. Lee Moffitt Cancer Center. PCR-SSCP and sequencing analysis was repeated for all polymorphism-positive samples.

PCR-restriction fragment length polymorphism (RFLP) analysis were subsequently developed to examine the prevalence of identified EH polymorphisms. Prevalences of genetic polymorphisms in the EH gene were measured in additional at least 50 healthy African Americans and Caucasians, respectively (Table 2).

Genotyping Assays for codons 43, 113 and 139 polymorphisms

One hundred sixty nine lung cancer patients and matched controls were screened for the presence of the EH codon 43 polymorphism by a PCR-RFLP analysis. Exon 2 of EH gene were PCR-amplified using 100ng EH intron 1 (5'-ggtcttcccctcatcttgc-3') and intron 2 (5'-cccggcccaaggtgcctt-3') -specific primers to generate a 229bp fragment. The standard PCR was performed in a 50□l reaction volume containing 50ng of genomic DNA, 10mM Tris-HCl, 50mM KCl, 1.5mM $MgCl_2$, 0.2mM of each of the dNTPs, and 2.0 units of *Taq* polymerase . The reaction mixtures underwent the following incubations: 1 cycle of 95°C for 2 min, 40 cycles of 94°C for 30sec, 51°C for 30sec, and 72°C for 30sec, followed by a final cycle of 10 min at 72°C. EH exon 2 PCR-amplified fragments were treated with *BstN1*, which recognizes the wild-type (EH 43^{Arg} allele) but not polymorphic EH sequence (EH 43^{Tyr} allele). Differences in RFLP patterns were detected after *BstN1* restriction enzyme digestion at 60°C for 16h using 10ul of PCR amplification. In addition to the polymorphic *BstN1* site at codon 43, an additional *BstN1* site is present within the EH exon 2 PCR-amplified product, serving as an internal control for restriction enzyme digestion for all EH codon 43 polymorphism analysis. Three banding patterns were observed by RFLP analysis: 133bp, 79bp and 19bp bands that corresponded to the EH homozygous wild-type (EH $43^{Arg}/43^{Arg}$) genotype, 133bp, 96bp, 79bp and 19bp bands that corresponded to the EH heterozygous (EH $43^{Arg}/43^{Tyr}$) genotype, and 133bp, and 96bp bands that corresponded to the EH $43^{Tyr}/43^{Tyr}$ homozygous polymorphic genotype, respectively. The genotyping assays for the EH codons 113 and 139 polymorphism were performed by PCR-RFLP analysis, described previously[17]. This analysis was repeated for 10% of

the specimens and selected PCR-amplified DNA samples (n=20) were examined by dideoxy DNA sequencing [23] to confirm EH genotyping results.

Statistical Analysis

The risks of lung cancer in relation to EH genotypes were estimated using conditional logistic regression to calculate ORs and 95%CIs. In this study, we designated the four possible EH alleles arising from the codons 113/139 polymorphism analysis as EH*1 ($EH^{113Tyr/139His}$), EH*2 ($EH^{113Tyr/139Arg}$), EH*3 ($EH^{113His/139His}$), and EH*4 ($EH^{113His/139Arg}$) (Fig. 1). Subjects were categorized into three groups based on the predicted activity of their EH genotype as described previously [8]: the low EH activity genotype (EH*3/EH*3), intermediate EH activity genotypes (EH*1/EH*1, EH*1/EH*3, EH*2/EH*3, EH*2/EH*4 and EH*3/EH*4) and high EH activity genotypes (EH*1/EH*2, and EH*2/EH*2). The chi-square test was utilized for the analysis of allelic frequencies. The Student's t-test (2-tailed) was used for comparing smoking (py) variable between cases and controls. The statistical computer software SPSS (ver. 11.5) was used to perform all statistical analysis[24].

Results

PCR-SSCP analysis and identification of EH polymorphisms

Genomic DNA samples from at 80 healthy subjects (40 Caucasians and 40 African Americans) were subjected to SSCP-PCR analysis (Fig. 2). Table 2 shows the location and characters of 11 polymorphisms identified in the samples we analyzed. Among the 11 polymorphisms detected, five polymorphisms resulted in either amino acid

substitutions, or silent, respectively, and a polymorphism was located in the intron 4 region. Among the five amino acid changing polymorphisms, three of them were identified in previous studies [6-8]. The allelic frequencies of codon 113 and 139 polymorphisms were similar to those observed in previous studies of both Caucasians and African Americans [4, 8, 12, 14, 25]. The allelic frequency for codon 43 polymorphism was reported as 0.08 in a small study [6] (n=26), but we observed significantly lower allelic frequency in our total control population (0.01, n=252).

Differences in EH allelic frequencies were observed between Caucasians and African American controls. Two polymorphisms, which are located in intron 4 ($p < 0.02$) and at codon 450 ($p < 0.01$) show significantly different allelic frequencies between two control groups. In addition, a significantly ($p < 0.0001$) lower frequency of the EH^{139Arg} allele was observed for Caucasians (0.15) as compared to African Americans (0.31), while the EH^{113His} allelic frequency was significantly ($p < 0.0001$) higher in Caucasians (0.40) as compared to African Americans (0.20; Table 2).

EH genotypes and risk for lung cancer

A total of 169 Caucasian lung cancer patients, and individually matched control subjects were entered into this study. The mean ages of cases and controls were 62, and 65, and 41% of subjects were female (Table 3). As expected, cases had a significantly higher level of cigarette consumption than controls ($p < 0.001$; Table 3). To determine whether the EH variants contributed to increased risk for lung cancer, we examined the prevalence of EH genotypes in lung cancer patients and compared with

control subjects. Significantly increased risk for lung cancer was observed for subjects with both the heterozygous EH $113^{His}/113^{Tyr}$ genotype (odds ratio [OR] = 2.8, 95% confidence interval [CI] = 1.3 – 6.1) and the homozygous EH $113^{Tyr}/113^{Tyr}$ genotype (OR = 2.4, 95% CI = 1.1 – 5.0; Table 3). No significant association between the codons 43 and 139 polymorphism and lung cancer risk was observed, although the data suggested the potential trend (Table 3).

Genotypes of individual subjects were determined by the combined data obtained from individual PCR-RFLP analysis of the codons 113 and 139 polymorphisms. Due to low allelic frequency, codon 43 polymorphism was not included for determining combined genotypes. Other than the EH*2/EH*3 versus EH*1/EH*4 genotypes, all EH genotypes could be distinguished by this analysis. All subjects exhibiting the EH*2/EH*3 or EH*1/EH*4 genotypes were considered to be EH*2/EH*3 because the prevalence of the EH*4 allele is low in the population (0.016) and the predicted phenotypes of both EH genotypes are same intermediate EH activity category based upon functional analysis [8]. In controls, the prevalence of the EH^{113His} variant was 0.40, while the prevalence of the EH^{139Arg} variant was 0.15. The prevalences of these polymorphisms among controls were followed Hardy-Weinberg equilibrium.

When subjects were stratified based upon predicted EH activity genotypes, a significant increase in risk for lung cancer was observed among subjects with both intermediate and high EH activity genotypes as compared to the low EH activity genotype (intermediate EH activity, OR=2.7, 95% CI = 1.3 - 5.9; high EH activity, OR=5.0, 95% CI

= 1.9 - 13.3; Table 3). A significant association was found between predicted EH activity and lung cancer risk with a dose–effect relationship (trend test: $p<0.018$).

To examine the relationship between EH genotypes and lung cancer risk by exposure to environmental risk factor, smoking, we stratified study subjects by predicted EH activity genotypes and smoking history (Table 4). Ever-smokers (i.e., ≥100 cigarettes lifetime) were categorized into two groups based upon lifetime smoking history divided at the median number of pack-years (42 py) of all smokers in the entire cohort. The differences in risk associated with the EH genotypes were not significantly modified by smoking history. We observed similar trends in both heavy and light smokers. Due to low number of cases, we cannot assess role of EH polymorphisms among never-smokers (data not shown). Significantly increased risk for lung cancer was observed for light smokers (< 42 py) with high EH activity genotypes (OR = 5.4, 95% CI = 1.03 – 28.6) and heavy smokers (≥ 42 py) with both the intermediate (OR = 3.4, 95% CI = 1.3 – 9.1) and high (OR = 7.6, 95% CI = 1.4 – 41.1) EH activity genotypes (Table 4). Among heavy smokers, a significant association was found between predicted EH activity and lung cancer risk with a dose–effect relationship (trend test: $p<0.004$).

Discussion

Genetic polymorphisms in the genes coding for tobacco carcinogen metabolizing enzymes may influence individual susceptibility to lung cancer. Although EH-induced hydrolysis is generally considered to represent a detoxification reaction because less toxic chemicals are usually produced, some trans-dihydrodiols generated from PAHs

are substrates for additional metabolic changes to highly toxic, mutagenic, and carcinogenic polycyclic aromatic hydrocarbon diol epoxides. For example, EH converts BaP-7,8-epoxide to BaP–7,8-di-hydrodiol, which is a critical intermediate metabolite in the BaP carcinogenic pathway and is formed predominantly via oxidation by the CYP1A1 [26]. Once formed, BaP-7,8-epoxide can either be detoxified by the GST family of phase II enzymes, or metabolized by EH to a diol intermediate (BaP-7,8-dihydrodiol), precursor to the ultimate carcinogenic BaP metabolite, BaP-7,8-diol-9,10-epoxide. Therefore, EH may play a significant role in the BaP-induced carcinogenic process.

Among several previous molecular epidemiological studies have been performed on EH codons 113 and 139 polymorphisms, two large lung cancer studies, Zhou et al.[27] reported significant association was found among squamous cell carcinoma cases, and similar results were observed in pooled analysis of eight studies[28]. In this study, we confirmed a significant association between EH polymorphisms and risk for lung cancer in Caucasians. This association is dose-dependent with significantly increased risk observed for subjects with predicted high EH activity genotypes. These results are consistent with a critical role for EH in tobacco-related cancer risk and in the metabolism of BaP-7,8-epoxide. Similar to previous studies [4, 14], the effect of EH activity on lung cancer risk was not significantly modified by smoking level in the present study.

The data from this study demonstrate several previously-unidentified polymorphisms in the EH gene. It has been reported that SSCP analysis can detect 80-90% of single nucleotide change in less than 400 bp DNA fragments. However, a recent systematic analysis of the SSCP technique has shown that sensitivity varied with the size of DNA fragment being analyzed, with the optimal size being 150 bp [29]. The

sensitivity of detecting a single base pair substitution was actually less that 60% when DNA fragments were about 400 bp. Since the PCR fragment sizes in present study range from 149 bp for exon 7 to 308 bp for exon 4 (Table 1), polymorphisms were likely detected with relatively high efficiency (>90%).

This is the first data for prevalence of newly-identified genetic polymorphisms in the EH gene in African Americans and Caucasians. As expected, prevalences of genetic polymorphisms found in African Americans are often significantly different with one among Caucasians. These data corresponded with the fact that significant differences of prevalences were observed with EH codons 113 and 139 in different races [4, 12]. We investigated potential roles of codons 43, 113 and 139 amino-acid altering polymorphisms among 11 polymorphisms identified from screening process. The justification of investigating rest of polymorphisms for lung cancer risk was weak because they are not likely affect activity of EH enzyme nor can assess because of lack of power. Although we could not assess an exact role of codon 43 polymorphism due to a low allelic frequency, marginal protective effect was observed ($p<0.061$). Therefore, codon 43 polymorphism may be worth for further investigation with larger cohort study.

For another important smoking-related lung disease, chronic obstructive pulmonary disease (COPD) was also linked with codon 113 EH polymorphism [30-32]. However, these associations were not observed in other studies [33-35]. Therefore, the role of EH polymorphisms in risk for pulmonary obstruction is not yet conclusive. Since this disease is associated with smoking and increased risk

4-6 fold for lung cancer [36], it would be interesting to investigate the role of EH polymorphisms in risk for lung cancer among subjects who have pulmonary obstructive disease.

In present study, only the coding region of the EH gene had been analyzed by SSCP analysis. Polymorphisms in promoter or intronic region may also play important roles in lung cancer risk. Further studies examining these regions will be required to explore this possibility.

In conclusion, the present study has identified several polymorphisms in the coding region of the EH gene and that predicted high activity EH genotypes are associated with increased risk for lung cancer in Caucasians. These results are supportive of previous studies implicating EH polymorphisms in lung cancer risk.

Acknowledgments

The authors thank Joseph Burton for his participation in the collection of clinical samples and questionnaire data, Nina Wadhwa, Meliza Roque and Dr. Jun Zhou for providing clinical samples and demographic information, Dr. Michael Gruidl for helpful discussions, and Susan Gray for her excellent technical assistance. These studies were supported by NIH Grants: CA91314 (JP), CA084973 supplement (JP), CA084973 (MT), and DE12206 (PL).

References:

1. McWilliams JE, Sanderson BJ, Harris EL, Richert-Boe KE, Henner WD. Glutathione S-transferase M1 (GSTM1) deficiency and lung cancer risk. *Cancer Epidemiol Biomarkers Prev* 1995;4(6):589-94.

2. Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. *Cancer Epidemiol Biomarkers Prev* 1997;6(9):733-43.

3. Lazarus P, Park JY. Metabolizing enzyme genotype and risk for upper aerodigestive tract cancer. *Oral Oncol* 2000;36(5):421-31.

4. London SJ, Smart J, Daly AK. Lung cancer risk in relation to genetic polymorphisms of microsomal epoxide hydrolase among African-Americans and Caucasians in Los Angeles County. *Lung Cancer* 2000;28(2):147-55.

5. Park JY, Muscat JE, Kaur T, Schantz SP, Stern JC, Richie JP, Jr., Lazarus P. Comparison of GSTM polymorphisms and risk for oral cancer between African-Americans and Caucasians. *Pharmacogenetics* 2000;10(2):123-31.

6. Gaedigk A, Spielberg SP, Grant DM. Characterization of the microsomal epoxide hydrolase gene in patients with anticonvulsant adverse drug reactions. *Pharmacogenetics* 1994;4(3):142-53.

7. Green VJ, Pirmohamed M, Kitteringham NR, Gaedigk A, Grant DM, Boxer M, Burchell B, Park BK. Genetic analysis of microsomal epoxide hydrolase in patients with carbamazepine hypersensitivity. *Biochem Pharmacol* 1995;50(9):1353-9.

8. Hassett C, Aicher L, Sidhu JS, Omiecinski CJ. Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants. *Hum Mol Genet* 1994;3(3):421-8.

9. Harrison DJ, Hubbard AL, MacMillan J, Wyllie AH, Smith CA. Microsomal epoxide hydrolase gene polymorphism and susceptibility to colon cancer. *Br J Cancer* 1999;79(1):168-71.

10. Lancaster JM, Brownlee HA, Bell DA, Futreal PA, Marks JR, Berchuck A, Wiseman RW, Taylor JA. Microsomal epoxide hydrolase polymorphism as a risk factor for ovarian cancer. *Mol Carcinog* 1996;17(3):160-2.

11. Lin P, Wang SL, Wang HJ, Chen KW, Lee HS, Tsai KJ, Chen CY, Lee H. Association of CYP1A1 and microsomal epoxide hydrolase polymorphisms with lung squamous cell carcinoma. *Br J Cancer* 2000;82(4):852-7.

12. Wu X, Gwyn K, Amos CI, Makan N, Hong WK, Spitz MR. The association of microsomal epoxide hydrolase polymorphisms and lung cancer risk in African-Americans and Mexican-Americans. *Carcinogenesis* 2001;22(6):923-8.

13. Yin L, Pu Y, Liu TY, Tung YH, Chen KW, Lin P. Genetic polymorphisms of NAD(P)H quinone oxidoreductase, CYP1A1 and microsomal epoxide hydrolase and lung cancer risk in Nanjing, China. *Lung Cancer* 2001;33(2-3):133-41.

14. Benhamou S, Reinikainen M, Bouchardy C, Dayer P, Hirvonen A. Association between lung cancer and microsomal epoxide hydrolase genotypes. *Cancer Res* 1998;58(23):5291-3.

15. Zhao H, Spitz MR, Gwyn KM, Wu X. Microsomal epoxide hydrolase polymorphisms and lung cancer risk in non- Hispanic whites. *Mol Carcinog* 2002;33(2):99-104.

16. To-Figueras J, Gene M, Gomez-Catalan J, Pique E, Borrego N, Corbella J. Lung cancer susceptibility in relation to combined polymorphisms of microsomal epoxide hydrolase and glutathione S-transferase P1. *Cancer Lett* 2001;173(2):155-62.

17. Park JY, Schantz SP, Lazarus P. Epoxide hydrolase genotype and orolaryngeal cancer risk: interaction with GSTM1 genotype. *Oral Oncol* 2003;39(5):483-90.

18. Omiecinski CJ, Aicher L, Holubkov R, Checkoway H. Human peripheral lymphocytes as indicators of microsomal epoxide hydrolase activity in liver and lung. *Pharmacogenetics* 1993;3(3):150-8.

19. Elahi A, Zheng Z, Park J, Eyring K, McCaffrey T, Lazarus P. The human OGG1 DNA repair enzyme and its association with orolaryngeal cancer risk. *Carcinogenesis* 2002;23(7):1229-34.

20. Benhamou S, Benhamou E, Flamant R. Lung cancer risk associated with cigar and pipe smoking. *Int J Cancer* 1986;37(6):825-9.

21. Ausubel FM, Brent R, Kingston R, Moore DD, Seidman JG, Smith JA. Current Protocols in Mol. Biol. Vol. 1, New York, John Wiley and Sons, 1988.

22. Park JY, Muscat JE, Ren Q, Schantz SP, Harwick RD, Stern JC, Pike V, Richie JP, Jr., Lazarus P. CYP1A1 and GSTM1 polymorphisms and oral cancer risk. *Cancer Epidemiol Biomarkers Prev* 1997;6(10):791-7.

23. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. *Proc Natl Acad Sci U S A* 1977;74(12):5463-7.

24. SPSS. SPSS base 11.5 for windows, User's guide. SPSS Inc. Chicago. 2003..

25. Jourenkova-Mironova N, Mitrunen K, Bouchardy C, Dayer P, Benhamou S, Hirvonen A. High-activity microsomal epoxide hydrolase genotypes and the risk of oral, pharynx, and larynx cancers. *Cancer Res* 2000;60(3):534-6.

26. Phillips DH. Fifty years of benzo(a)pyrene. *Nature* 1983;303(5917):468-72.

27. Zhou W, Thurston SW, Liu G, Xu LL, Miller DP, Wain JC, Lynch TJ, Su L, Christiani DC. The interaction between microsomal epoxide hydrolase polymorphisms and cumulative cigarette smoking in different histological subtypes of lung cancer. *Cancer Epidemiol Biomarkers Prev* 2001;10(5):461-6.

28. Lee WJ, Brennan P, Boffetta P, London SJ, Benhamou S, Rannug A, To-Figueras J, Ingelman-Sundberg M, Shields P, Gaspari L, Taioli E. Microsomal epoxide hydrolase polymorphisms and lung cancer risk: a quantitative review. *Biomarkers* 2002;7(3):230-41.

29. Sheffield VC, Beck JS, Kwitek AE, Sandstrom DW, Stone EM. The sensitivity of single-strand conformation polymorphism analysis for the detection of single base substitutions. *Genomics* 1993;16(2):325-32.

30. Yoshikawa M, Hiyama K, Ishioka S, Maeda H, Maeda A, Yamakido M. Microsomal epoxide hydrolase genotypes and chronic obstructive pulmonary disease in Japanese. *Int J Mol Med* 2000;5(1):49-53.

31. Sandford AJ, Chagani T, Weir TD, Connett JE, Anthonisen NR, Pare PD. Susceptibility genes for rapid decline of lung function in the lung health study. *Am J Respir Crit Care Med* 2001;163(2):469-73.

32. Rodriguez F, Jardi R, Costa X, Juan D, Galimany R, Vidal R, Miravitlles M. Detection of polymorphisms at exons 3 (Tyr113-->His) and 4 (His139-- >Arg) of the

microsomal epoxide hydrolase gene using fluorescence PCR method combined with melting curves analysis. *Anal Biochem* 2002;308(1):120-6.

33. Yim JJ, Yoo CG, Lee CT, Kim YW, Han SK, Shim YS. Lack of association between glutathione S-transferase P1 polymorphism and COPD in Koreans. *Lung* 2002;180(2):119-25.

34. Yim JJ, Park GY, Lee CT, Kim YW, Han SK, Shim YS, Yoo CG. Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1. *Thorax* 2000;55(2):121-5.

35. Zhang R, Zhang A, He Q, Lu B. [Microsomal epoxide hydrolase gene polymorphism and susceptibility to chronic obstructive pulmonary disease in Han nationality of North China]. *Zhonghua Nei Ke Za Zhi* 2002;41(1):11-4.

36. Tockman MS, Anthonisen NR, Wright EC, Donithan MG. Airways obstruction and the risk for lung cancer. *Ann Intern Med* 1987;106(4):512-8.

Table 1. Primer sequences, annealing temperatures, and PCR fragment lengths used for SSCP analysis.

Exon	Sense Primer	Antisense Primer	Anneal Temp. (°C)	Size of exon (bp)[1]	Size of PCR product (bp)
2	ggtcttcccctcatcttgc	cccggcccaaggtgcctt	54	182	229
3	ggtcctgaattttgctccag	ggctggcgttttgcaaacat	51	181	223
4	tgactgtgctctgtccccc	caccgggcccacccttgg	57	228	308
5	cctttccccatcactgcc	caccccaacagtgacctc	53	130	170
6	gcccctctctctgccttc	ggacccctgagcactctc	55	209	249
7	ttgctccccgctcccgcc	ggggcaaaccagggcctc	57	109	149
8	ctggctgccctttgtcacac	ttcgtggagcccttgtctg	54	126	268
9	gtcggctctttcacttcc	aagcctggagggcacttg	51	202	275

[1] Sizes of exons 2 and 9 indicate coding region

Table 2. Prevalences of polymorphisms in EH gene in Caucasian and African American controls

Location	Codon	Sequence change	Amino acid change	Allelic frequencies	
				Caucasians	African Americans
Exon 2	43[1]	G<C	Arg<Thr	0.01 (4/336)[2]	0.01 (2/168)
Exon 3	113[1]	T<C	Tyr<His	0.40 (136/336)[3]	0.20 (54/274)
Exon 3	119	G<A	Silent (Lys)	0.13 (25/188)	0.16 (16/100)
Intron 4	+34[1]	G<A		0.10 (16/166)[3]	0.02 (2/102)
Exon 4	139[1]	A<G	His<Arg	0.15 (50/334)[3]	0.31 (89/286)
Exon 4	149	C<T	Silent (Gly)	0.01 (2/166)	0.01 (1/100)
Exon 4	195	T<C	Ser<Pro	0.006 (1/168)	0.01 (1/102)
Exon 6	284	C<T	Silent (Pro)	0.08 (11/146)	0.08 (9/108)
Exon 8	357	T<C	Silent (Asn)	0.11(18/168)	0.14(20/142)
Exon 8	382	G<T	Trp<Leu	0.006 (1/168)	0.01 (1/100)
Exon 9	450	G<C	Silent (Ser)	0.06(10/168)[3]	0(0/100)

[1] Previously reported polymorphisms
[2] Assay sample size (number of alleles detected/number of alleles screened).
[3] Allelic frequencies were significantly different between African American and Caucasian controls.
SPSS. SPSS base 11.5 for windows, User's guide. SPSS Inc. Chicago. 2003.

Table 3. Demographic information of subjects, EH polymorphisms, predicted EH activity genotypes and lung cancer risk.

			Cases	Controls	
n			169	169	
mean age (range)			65.2 (36-83)	61.7 (31-86)	
Sex (M/F)			100/69	100/69	
Smoking [mean ± SD (py)][1]			53.1 ± 31.7	18.8 ± 25.0	
Location	Codon	genotypes	Cases	Controls	OR (95%CI)[2]
Exon 2	43	arg/arg	169 (100)[3]	164 (98)	1.0 (referent)
		arg/thr	0 (0)	4 (2)	NA[4]
Exon 3	113	his/his	25 (15)	38 (23)	1.0 (referent)
		tyr/his	67 (41)	60 (36)	2.8 (1.3-6.1)
		tyr/tyr	77 (44)	70 (41)	2.4 (1.1-5.0)
Exon 4	139	his/his	115 (68)	120 (72)	1.0 (referent)
		arg/his	47 (28)	44 (26)	1.6 (0.9-2.8)
		arg/arg	7 (4)	3 (2)	2.4 (0.5-11.4)
Predicted EH activity genotypes[5]			Cases	Controls	OR (95%CI)[2]
	low		20 (12)	32 (19)	1.0 (referent)[6]
	intermediate		117 (69)	114 (69)	2.7 (1.3-5.9)
	high		32 (19)	20 (12)	5.0 (1.9-13.3)

[1] Smoking consumption was significantly higher ($p < 0.001$) in cases as compared to controls.
[2] ORs were calculated by adjusting for sex, age, and smoking (py).
[3] Numbers in parenthesis denote percentages.
[4] p=0.061 by Fisher exact test.
[5] (high), subjects with either the EH*1/EH*2, or EH*2/EH*2 genotypes; (intermediate), subjects with either the EH*1/EH*1, EH*1/EH*3, EH*2/EH*3, EH*2/EH*4 or EH*3/EH*4 genotypes; (low), subjects with either the EH*3/EH*3 genotype.
[6] Significant increase in predicted high-risk genotypes as determined by χ^2-trend test ($p = 0.018$).

Table 4. Lung cancer risk for predicted EH activity genotypes stratified by smoking.

Smoking level	Predicted EH Activity genotypes	Cases	Controls	OR (95%CI)[1]
LS (<42py)	low	5 (10)[2]	11 (15)	1.0 (referent)[3]
	intermediate	36 (69)	52 (73)	2.0 (0.5-7.3)
	high	11 (21)	8 (12)	5.4 (1.03-28.6)
HS (≥42py)	low	14 (14)	11 (37)	1.0 (referent)[4]
	intermediate	71 (70)	17 (57)	3.4 (1.3-9.1)
	high	17 (16)	2 (6)	7.6 (1.4-41.1)

[1] ORs were calculated by adjusting for sex, and age
[2] Numbers in parenthesis denote percentages.
[3] Increase risk in predicted high-risk genotypes as determined by χ^2-trend test ($p = 0.08$).
[4] Significant increase in predicted high-risk genotypes as determined by χ^2-trend test ($p = 0.004$).

Figure Legend

Fig. 1. Schematic diagram of epoxide hydrolase alleles and their corresponding polymorphisms at codons 113 and 139.

Fig. 2. Detection of polymorphisms in exon 4 by SSCP analysis. Genomic DNA was used as templates in PCR designed for the amplification of the EH exon 4. Shown is a representative analysis of SSCP analysis of EH exon 4 for 21 individual DNA samples. The band A indicates EH allele with intron 4 polymorphism. Band B indicates allele with intron 4 and codon 139 polymorphism. Band C indicates wild type allele.

Abstract, presented at Gordon Research conferences. New Frontiers in Cancer Detection & Diagnosis. March 10-15, 2002. Ventura, CA
Chen, L., Lazarus, P., Tockman, M. and Park, J. (2002)
"Epoxide hydrolase polymorphisms and risk for lung and oral cancer"

ABSTRACT

EH catalyzes the conversion of benzo[a]pyrene (BaP)-7,8-epoxide to BaP-7,8-diol, the direct precursor metabolite of BaP-7,8-diol-9,10-epoxide, the ultimate carcinogen of BaP. Two EH genetic polymorphisms have been described, one in exon 3 at codon 113 (Tyr>His) and another in exon 4 at codon 139 (His>Arg). To assess the potential role of EH polymorphisms in risk for lung and oral cancers, PCR-RFLP analysis was used to identify EH genotypes in buccal cell DNA specimens isolated from Caucasian lung (n=86) and oral (n=142) cancer patients and controls (n=142). The prevalence of the EH exon 3 (EH^{113His}) and exon 4 (EH^{139Arg}) polymorphic variants in controls were 0.37 and 0.19, respectively. Significantly decreased risk for lung and oral cancer was observed for Caucasian subjects possessing predicted low-activity EH genotypes 113(his/ his) as determined by multiple regression analysis adjusting for age, sex, smoking, and alcohol consumption (oral cancer OR=0.5; 95%CI=0.3-0.96; lung cancer , OR=0.5, 95%CI=0.2-1.0). No association between exon 4 polymorphism and lung and oral cancer risk was observed. These results suggest that, (i) EH exon 3 polymorphism may play an important role in lung and oral cancer risk in Caucasians, (ii) BaP-(7,8)-epoxide activation as well as detoxification pathways are important determinants of lung and oral cancer risk.

Abstract presented at the annual AACR meeting

Park, J., and Lazarus, P (2001) Epoxide hydrolase polymorphism and oral cancer risk: correlation with the GSTM1-null genotype. Proc. Amer. Assoc. cancer res. 40:566, 2001. The 92nd Annual Meeting American Association for Cancer Research. March 24-28, 2001. New Orleans, LA.

ABSTRACT

EH catalyzes the conversion of benzo[a]pyrene (BaP)-7,8-epoxide to BaP-7,8-diol, the direct precursor metabolite of BaP-7,8-diol-9,10-epoxide, the ultimate carcinogen of BaP. Two EH genetic polymorphisms have been described, one in exon 3 at codon 113 (Tyr>His) and another in exon 4 at codon 139 (His>Arg). To assess the potential role of EH polymorphisms in risk for oral cancer, PCR-RFLP analysis was used to identify EH genotypes in buccal cell DNA specimens isolated from Caucasian and African American oral cancer cases and controls. The prevalence of the EH exon 3 (EH^{113His}) and exon 4 (EH^{139Arg}) polymorphic variants in controls were 0.37 and 0.19 in Caucasians (n=192), and 0.20 and 0.32 in African Americans (n=124), respectively. Significantly increased risk for oral cancer was observed for Caucasian subjects possessing predicted high-activity EH genotypes [113(Tyr/ His)/139(Arg/Arg), 113(Tyr/ Tyr)/139(Arg/Arg), and 113(Tyr/Tyr)/139(Arg/His)] as determined by multiple regres-sion analysis adjusting for age, sex, smoking, and alcohol consumption (OR=2.0; 95%CI=1.1-3.5). This increased risk was observed in heavy-smokers ($\geq$ 35 pack-years; OR=3.3, 95%CI=1.2-9.1) but not in light smokers (<35 pack-years; OR=0.9, 95%CI=0.3-2.5). No association with oral cancer risk was observed in African Americans even after stratification by smoking dose. Although an association between the GSTM1 null genotype and risk for oral cancer was not observed for Caucasians in previous studies (1), significant increases in oral cancer risk were observed in the present study for Caucasian subjects who had the combined GSTM1 (0/0)/EH high activity genotypes (ORadj=3.0; 95%CI=1.2-7.2). No association between high activity EH genotypes and oral cancer risk was observed in Caucasian subjects who were GSTM1-positive (ORadj=1.2; 95%CI=0.5-2.6). These results suggest that, (i) combined high risk EH and GSTM1 genotypes play an important role in oral cancer risk in Caucasians, (ii) BaP-(7,8)-epoxide activation as well as detoxification pathways are important determinants of oral cancer risk, and (iii) stratification by multiple geno-types is important for accurate risk assessment analysis in specific racial groups.

APPENDIX B

Automated Quantified Screening for Melanoma

Dmitry G. Goldgof, Ph.D.
Sudeep Sarkar, Ph.D.
Wayne C. Cruse, MD

Automated Quantified Screening for Melanoma

Dmitry G. Goldgof, Ph.D.
Sudeep Sarkar, Ph.D.
Wayne C. Cruse, MD

INTRODUCTION

Our long term goal was to design an imaging device that could be used by patients for in-home screening for melanoma and to refer potential melanoma cases to experts. The hypothesis was that information from color images in the visible spectrum range along with collateral information about various risk factors is sufficient for a high sensitivity diagnosis. To keep the costs down, this device was envisioned to use off-the-shelf non-contact imaging techniques, along with sophisticated image analysis algorithms and a probabilistic knowledge base, to suggest the probability that a lesion is a melanoma. Unlike other image analysis based melanoma detection algorithms the proposed approach used knowledge of the imaging and illumination geometry to automatically correct for artifacts, such as shading, to arrive at "true" color before computing the ABCD attributes of the skin lesion, such as its asymmetry (A), border irregularity (B), color variation (C), and diameter (D). The architecture of this system differs from other approaches in its use of a sophisticated probabilistic expert system to integrate information about various risk factors, along with computed ABCD attribute to arrive at a probability of the lesion under consideration being a melanoma. The underlying hypothesis is that the use of collateral information about the risk factors will help improve the sensitivity and specificity of screening.

BODY:

Over the last two years, our work has been mostly in the development of the computer algorithms that would quantify the ABCD aspects of skin melanoma and in deciding upon the hardware imaging configuration that would be appropriate. The research accomplishments associated each task outlined in our proposal are itemized below.

Task 1: Design the screening device and construct its components

1. Decide on the hardware configuration. How many lights are necessary for imaging a skin melanoma? What kinds of lights are best? What should be the spectral distribution of the light sources? What arrangement of camera and lights is the most convenient?
2. Adapt and refine the color-texture characterization and segmentation algorithms that we have developed for burn scar for skin melanoma detection.
3. Implement the ABCD criteria for melanoma detection.

Task-1 Accomplishments:

- We have thoroughly investigated the hardware configuration that would be best suitable for the most flexible imaging. Instead of the Cyberware range scanner, which is not portable, we identified the Minolta range scanner, which is shown in Figure 1. This scanner has a bit lower range resolution, about 0.2mm in depth, than the Cyberware scanner, which had 0.1mm depth resolution. However, the Minolta scanner is portable and can be flexibly positioned on tripod to image skin lesions at difficult-to-image locations. We have also identified the light source to use along this scanner that would be best for skin color tones.

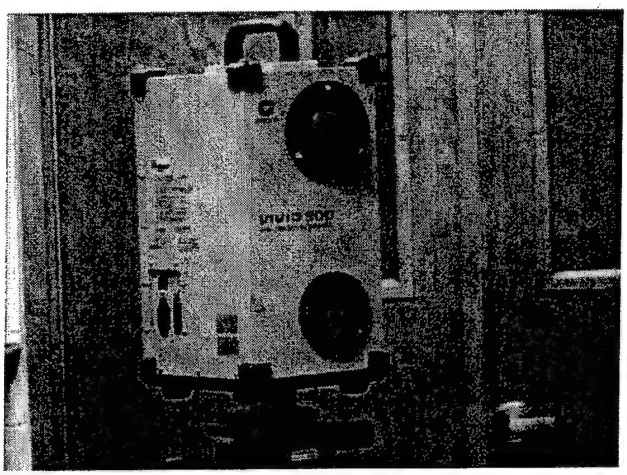
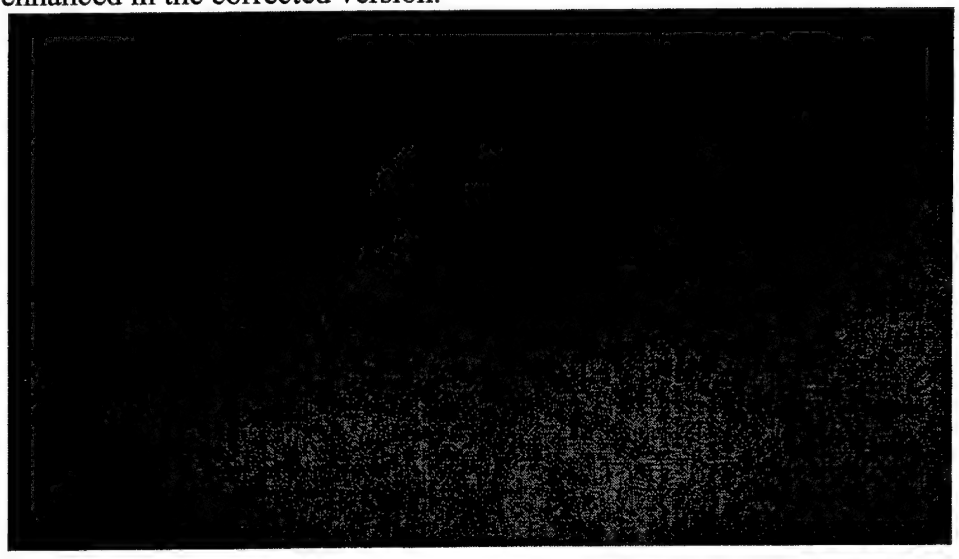


Figure 1 Minolta Range Scanner

- We have design/refined color correction and calibration algorithm to work with the Minolta scanner. Figure 2 shows an example of the color correction algorithm at work. Notice how the moles labeled 1 and 5, which are towards the shaded portions for the image are enhanced. The underlying texture of the skin is also enhanced in the corrected version.



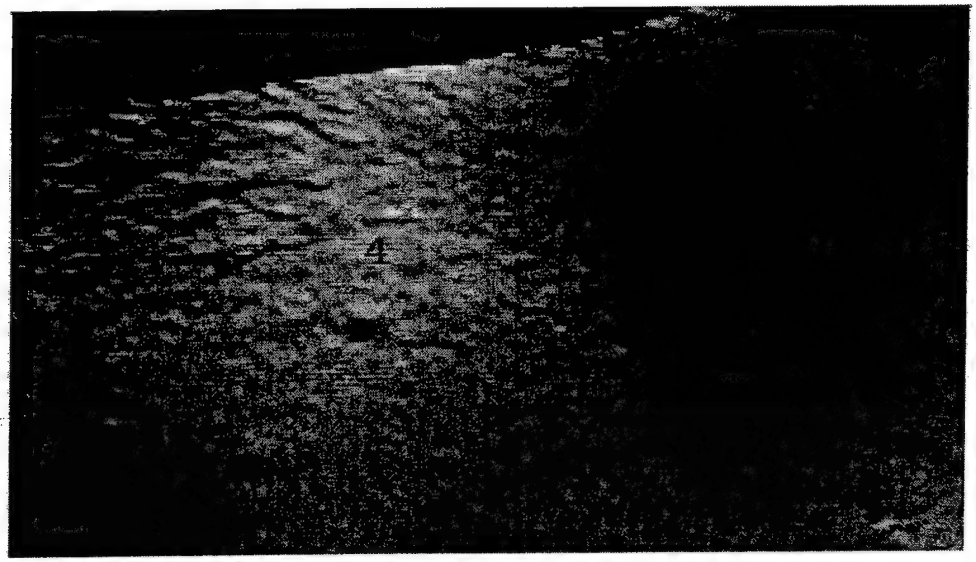
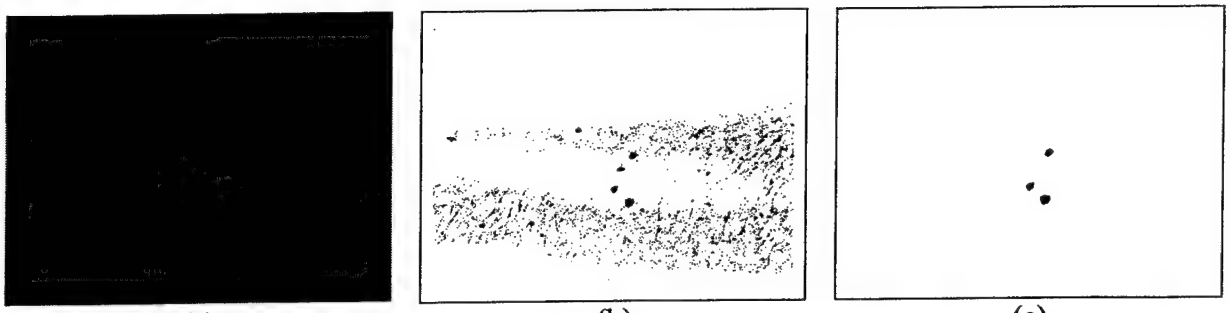


Figure 2 Top: Raw color image without compensating for illumination. Bottom: Color corrected image.

- We have implemented segmentation and image feature extraction algorithms that approximate the ABCD criteria for melanoma detection. The segmentation procedure has five steps

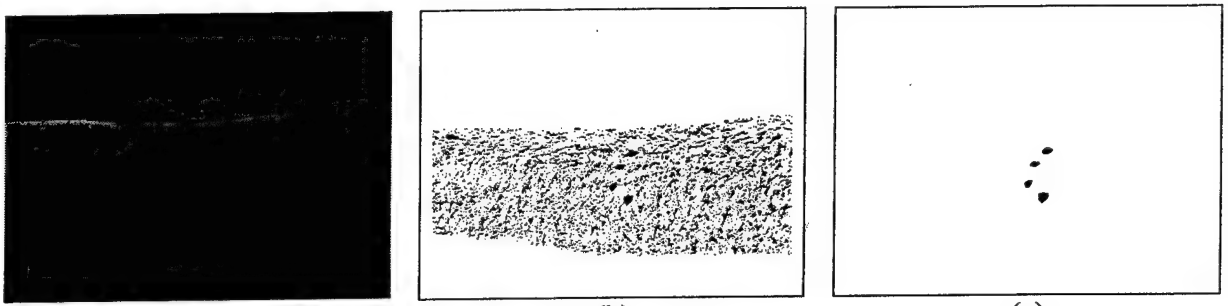
    - Transforming a color image into grey image using HSI color space
    - Gaussian smoothing of the intensity image to suppress noise;
    - Morphological closing operation to erase hair from the image;
    - Binarization using threshold based on the image histogram;
    - To distinguish benign lesion from melanoma we quantify the ABCD rule into 43 features as follows. Lesion symmetry is captured using
        - Standard deviation of the distance from the center of gravity to the boundary.
        - Ratio of the minimal distance of the boundary from the center to the maximal one.
        - The difference in perimeter and area between the Left and right halves of the two dimensional image of the lesion as determined by dividing the lesion horizontally and vertically into two parts across the center of gravity.
        - Quadrant shape similarity as captured by comparing the four parts of the lesion created by dividing into four equal quadrants and computing the ratio of the area and perimeter
        - The roundness of the spot is evaluated by the central moments of the first, second, and third order and the compactness. Compactness is computed as a ratio of the area of the spot to its perimeter.
    - The color aspects of the lesion are captured using the following features.

        - The color of a benign lesion is usually evenly spread over the lesion, while the color of the melanoma is uneven. There can be spots of the different color. The first color feature is the standard deviation and the minimal to maximal ratio of the three color channels. The standard deviation of the red, green, and blue color bands is computed. Then, we transform RGB (Red, Green, Blue) to HSI (Hue, Saturation, Intensity) and compute the same features for the intensity, saturation, and hue components.
        - The ratio of the light area of the spot to the dark area. The problem here is to choose the threshold. We choose the threshold manually for now by finding the minima on the histograms of the three color bands. This will be automated in future.
        - We count the number of dark spots in the lesion. This feature also demands a threshold be chosen the same way as before. Before counting the number of dark spots, the image should be transformed to the grey image and smoothed with the Gaussian filter to remove noise.
        - We consider rays with 1 degree of separation from the center of gravity to the boundary and selected only those lines that have number of pixels equal to or more than the half of the number of pixels in the diameter of the lesion. Then we count the number of local minima along each line for each color band.
        - The average gradient on the boundary is another feature. A six-pixel wide mask is applied to the boundary of a spot, and the value of the gradient in each pixel in the mask is computed. This feature shows us the transition from the spot to the normal skin. Benign lesions usually have a sharp border whereas the border of a melanoma can be uneven.
    - The border irregularity is captured by the first eight central moments of the boundary and the diameter is defined as the maximum distance between two boundary points.
- We classify the identified lesions using decision trees (C4.5) and support vector machine. Using microscope images of melanoma that are publicly available we can achieve about 86% to 90% using the 43 features and using a leave-out-one strategy for training and testing.
- We worked on the problem with automatic location and segmentation of skin lesions so as to have a completely automatic end-to-end system of correction, detection, and classification. Our data used for this consists of 11 images and 65 lesions taken from various skin areas of the PIs involved in the study. This is not patient data. The images were taken at different time periods as well as under different light conditions. All of the images were taken with the Minolta 3D Digitizer scanner. The light source is a sun light spectrum light that is designed to best emulate the sun light.



(a) (b) (c)

Figure 3 - Segmentation results with original color images. (a) Original Image, (b) Intermediate segmentation, (c) Final Segmentation.



(a) (b) (c)

Figure 4 – Segmentation results with color corrected images. (a) Color corrected image, (b) intermediate segmentation, (c) Final segmentation.

- The specific steps of the automated detection process are as follows. Please refer to Figure 3 for a sample original image and to Figure 4 for color corrected one. It is performed in two steps; the first one involving a global process and the second one being a local process. The global process used to hone in onto the lesions of interest are as follows:

    - Perform color correction
    - Perform local histogram segmentation using 2 thresholds calculated specifically for that region to result in segmentation as shown in Figure 3 (b) and Figure 4 (b).
    - Median filter the image to eliminate artifacts due to hair
    - Perform morphological closing to remove small components that will not be considered lesions
    - Perform connected components analysis to determine the actual location of the lesions

  The local process used to refine the segmentations involved the following steps

    - Use the color image and the location information to create masks around the individual lesions and calculate the projected distribution of all pixels in RGB space
    - Fit two Gaussians for the segmentation in the projected space
    - Use the above threshold to separate normal skin from lesion

- Apply medium filter to reduce over-segmentation errors
- Apply morphological opening to compensate for the median filter
- Producing the segmented binary image containing all lesions as shown in Figure 3 (c) and Figure 4 (c).

- We evaluated the effectiveness of the automated lesion detection algorithm at two level: at lesion count level (how many lesions are correctly detected and how many are missed?) and at pixel level (what percentage of a lesions is correctly marked as being a lesion pixel and what percentage of pixels are falsely marked as being lesion pixles?) These two evaluate the global and local steps of the segmentation procedure outlined above. Table 1 and Table 2 list the detection results at lesion level for each image for original and color corrected images, respectively. Table 3 and Table 4 list the pixel level detection performance for each image for original and color corrected images, respectively. The overall performance can be summarized as follows

  - With color correction we achieve 100% detection rate with 13% false alarms. While the detection rate for original image is 72% for a 9% false alarm. Note that detection rate is more important at this level.
  - The pixel level detection, given that we have correctly detected a lesions is around 81% and 83% for the original and corrected image. That is based on pixel by pixel comparison against a human segmented ground truth. What was interesting is that we achieve the same average in both color corrected and original data, however the variance in the test results were significantly different. The color corrected images show three times better variance in detection rate as the ones of the original images. Out set of data is not significant, but we can still conclude the accuracy of the corrected images contribute definitely for the success of locating and segmenting of skin lesions.

ORIGINAL IMAGES				
Image #	Gd truth lesions #	Detected lesions #	False positive #	False negative #
1	8	8	0	0
2	12	9	1	4
3	9	9	0	0
4	7	7	1	1
5	3	5	2	0
6	5	1	0	4
7	4	4	1	1
8	4	2	0	2
9	5	4	1	2
10	4	3	0	1
11	4	1	0	3
Totals:	65	53	6	18
Rates:		72%	9%	27%

Table 1 – Location Table for Original Images

COLOR CORRECTED IMAGES				
Image #	Gd truth lesions #	Detected lesions #	False positive #	False negative #
1	8	9	1	0
2	12	12	0	0
3	3	4	1	0
4	2	2	0	0
5	3	4	1	0
6	5	6	1	0
7	4	5	1	0
8	4	5	1	0
9	5	6	1	0
10	4	4	0	0
11	4	4	0	0
Totals:	54	61	7	0
Rates:		100%	13%	0%

Table 2 – Location Table for Color Corrected Images

ORIGINAL IMAGES									
Image #	# Lesions	TP # pixs	FP # pixs	FN # pixs	Total # pixs		% TP/total	% FP/total	% FN/total
1	8	1119	386	197	1316		0.850304	29.33%	14.97%
2	9	1440	616	467	1907		0.755113	32.30%	24.49%
3	6	886	36	337	1223		0.724448	2.94%	27.56%
4	6	886	36	337	1223		0.724448	2.94%	27.56%
5	3	378	35	53	431		0.87703	8.12%	12.30%
6	1	76	0	37	113		0.672566	0.00%	32.74%
7	3	288	47	30	318		0.90566	14.78%	9.43%
8	2	263	20	70	333		0.78979	6.01%	21.02%
9	3	336	13	33	369		0.910569	3.52%	8.94%
10	3	353	49	42	395		0.893671	12.41%	10.63%
11	1	128	7	21	149		0.85906	4.70%	14.09%
Totals:	**45**	**6153**	**1245**	**1624**	**7777**	**Averages:**	**81.48%**	**10.64%**	**18.52%**

Table 3 – Rates per image for Original Images

COLOR CORRECTED IMAGES									
Image #	# Lesions	TP # pixs	FP # pixs	FN # pixs	Total # pixs		% TP/total	% FP/total	% FN/total
1	8	1042	289	274	1316		79.18%	21.96%	20.82%
2	12	1599	293	446	2045		78.19%	14.33%	21.81%
3	3	451	22	71	522		86.40%	4.21%	13.60%
4	2	300	91	25	325		92.31%	28.00%	7.69%
5	3	352	39	79	431		81.67%	9.05%	18.33%
6	5	566	142	98	664		85.24%	21.39%	14.76%
7	4	382	65	48	430		88.84%	15.12%	11.16%
8	4	463	33	153	616		75.16%	5.36%	24.84%
9	5	492	32	113	605		81.32%	5.29%	18.68%
10	4	438	97	64	502		87.25%	19.32%	12.75%
11	4	479	47	127	606		79.04%	7.76%	20.96%
Totals:	**54**	**6564**	**1150**	**1498**	**8062**	**Averages:**	**83.15%**	**13.80%**	**16.85%**

Table 4 – Rates per image for Color Corrected Images

- **Task 2**: Collect melanoma and non-melanoma image data from 10 to 15 patients.

We have not yet been able to collect data from real patient. The IRB was first approved by USArmy only in May 2002. But, Dr. Reintgen, who would have helped us in recruiting patients, has left Moffitt in Sept 2002. We then identified Drs De Conti and Glass as our new contacts for patient recruitment. In addition, in Fall 02 we acquired a new range scanner, which was portable and more suitable for on-site data collection at the clinics. With our old scanner, the patients had to travel to our department. We submitted the change in protocol involving both the change in personnel and change in equipment to USF IRB, which approved the changes in Nov 2002. The CIP application has since been under processing at US Army. It only late Aug-2003 that we received request for more information.

- April, 2001: Submitted to USF IRB and Moffitt IRB
- April, 2001: Approved by Moffitt IRB
- July 6, 2001: Approved by USF IRB
- Oct 27, 2001: Extensive suggested revisions from US Army - HSRRB
- Jan 15, 2002: Revised version submitted to US ARMY-HSRRB
- Feb 7, 2002: Suggested revisions from US ARMY-HSRRB
- Feb 14, 2002: Revised version submitted to US ARMY – HSRRB
- Feb 20, 2002: Minor revisions from US ARMY – HSRRB
- Feb 26, 2002: Final US ARMY – HSRRB revised version submitted to USF – IRB
- March 7, 2002: Approval of final US-ARMY suggested revisions from USF –IRB
- March 26, 2002: USF – IRB approval submitted to US ARMY-HSRRB
- May 31, 2002: Approved by US ARMY – HSRRB
- Aug, 2002: Change in personnel and equipment submitted to USF-IRB
- Nov, 2002: Changes approved by USF
- Dec, 2002: Change in Protocol submitted to US Army-HSRRB
- Aug, 2003: Request for more information by US Army-HSRRB

- **Task 3**: Collect expert knowledge to design the probabilistic Bayesian network that will integrate background information such as heredity, life style, and ABCD attributes computed from images and arrive at the probability that the skin lesion under consideration is potentially malignant.

Due to lack of real patient data this task could not be undertaken.

KEY RESEARCH ACCOMPLISHMENTS:

We have mostly worked on algorithm development and device design. Our analysis has been mostly of parts of the systems and not the system as a whole. Even though our achievements in this stage are mostly on the algorithms that locate and segment the lesions from images, we greatly contributed toward the completion of the system as a

whole. We have completed this phase of the research and have results to show the advantage using color correction in our further processing.

REPORTABLE OUTCOMES:

- Yelena Mukomel, who has worked on the design of the computer algorithms, will defend her Master's thesis in Oct 2002.
- We were successful in getting an equipment grant from the National Science Foundation (NSF Grant EIA 0130768) to buy the Minolta range scanner that will be used in this study.
- Krassimir Ivanov, who has worked on the design of the computer algorithms, will defend his Master's thesis in Oct 2002.
- All the necessary hardware and software has been acquired and developed

CONCLUSIONS:

In summary, our progress although slow, partly due to reasons beyond our control, has been steady. Most of the work has been into the components (algorithms, cameras, lights etc) that would form the system. We have completed all the parts of the automatic location and segmentation of skin lesions. The work accomplished has been mostly on the design and implementation of the algorithms and procedures that handle the process described above. This progress with significantly improve the future processing of the data for classification on the lesions. We still would like to continue the research in this aspect when more data is available and when different variation of patient population is scanned for the testing of the algorithms and procedures. We are hoping to have the opportunity to continue the research in this direction in the near future.

References:

[1] P. Carli and B. Glannotti, "Dermatoscopy in the Diagnosis of Pigmented Skin Lesions: A New Semiology for the Dermatologist", *Journal of the European Academy of Dermatology and Venereology*, 14, pp. 353-369, 2000.

[2] Minolta Co., Ltd., http://www.minolta.com, 2003.

[3] C. A. Morton and R. M. Mackie, "Clinical Accuracy of the Diagnosis of Cutaneous Malignant Melanoma", *British Journal of Dermatology*, 138, pp.283-287, 1998.

[4] Y. Mukomel, MS Thesis: "Analysis of Lesions in Three-Dimensional Skin Images", Oct 2002.

[5] M. W. Powell, Ph.D. Dissertation: "Towards Objective Color from Images", Nov 2000.

APPENDIX C

Adaptive Computer Assisted Diagnosis (CAD) Method for Lung Nodule Early Detection

Craig Beam, PhD
Wei Qian, PhD

Adaptive Computer Assisted Diagnosis (CAD) Method for Lung Nodule Detection

CAD vs. Human Accuracy in the Interpretation of Screening Mammograms : A Pilot Study

Principal Investigators: C Beam, PhD and W. Qian, PhD

Dr. Qian and team have successfully created and validated a CAD algorithm on an independent set of digitized mammograms selected from the "VIDI" research program (cases were acquired under R01CA74110). In addition, Dr. Qian and team have successfully applied the CAD algorithm to another set of 130 cases. These latter cases are composed of screening and diagnostic mammograms and represent breast cancer, benign breast disease and normal mammographic features. Hence, the CAD data have been collected.

Analysis of the accuracy of the CAD system compared to human observers is now underway. An initial summary of the performance of CAD with respect to callback rates in screening is provided below. If we assume that the case is called-back whenever the CAD finds either a calcification or a mass, we observe that the system has screening "sensitivity" (correctly calling

DX truth	1			
Count of Mass	Mass			
Calc	0	1	.	Grand Total
0			1	1
1	2	15	7	24
.	2			2
Grand Total	4	15	8	27

= MISSES

Cancer
3 misses of 27

DX truth	0			
Count of M	Mass			
Calc	0	1	.	Grand Tota
0	1	2		3
1	6	17	4	27
Grand Tota	7	19	4	30

Normal
29 FP out of 30

back a case with cancer) of 24/27=89% and screening False Positive Probability of 29 out of 30 (97%). From the ROC perspective, a diagnostic test should always have Sensitivity exceeding False Positive-or else it performs less than that expected by chance. We observe that, at this point in analysis, the CAD performs less accurately than the toss of a fair coin.

Obviously, the CAD system is not meant to replace the screening radiologist but to assist and the previous analyses point out the need to measure the performance of the CAD against the presence or absence of calcification and mass. In preparation to that analysis, Dr. Beam and his team are registering the location of lesions on each of the cases using the original radiologist's report as the gold standard. That step is expected to be accomplished by the end of November 2003. We then will reanalyze the performance of CAD in a manner similar to the above and compare this lesion-specific performance against that of the 110 radiologists who participate in the VIDI studies. We anticipate that analysis will be completed by the end of the year and will form the foundations of a competing continuation application to be submitted March 1, 2004.

APPENDIX D

Breast Cancer Screening in High-Risk Women: Comparison of magnetic resonance imaging (MRI) with mammography

Robert A. Clark, M.D.

"Breast Cancer Screening in High-Risk Women: Comparison of Magnetic Resonance Imaging (MRI) with Mammography"
Proposal Log Number 01134008, Award Number DAMD17-01-2-0056
HSRRB Log Number A-10991.2
Principal Investigator- Robert A. Clark, M.D.

1. INTRODUCTION

This study compares MRI to mammography for screening of preclinical breast cancer in high-risk women. This pilot study poses the following hypotheses: 1) Screening with MRI for preclinical breast cancer in high-risk women is more accurate than with mammography; 2) The sensitivity and specificity of screening MRI are greater than that of screening mammography; and 3) the predictive values of screening MRI are greater than that of screening mammography. The aim of this study is to acquire preliminary data and determine the feasibility toward design of a larger, multiple year, multi-center trial of MRI screening. This study will determine if preliminary data warrant such a trial. Current screening recommendations for high-risk young women are not proven and are probably inadequate. If MRI is shown to be a better screening modality than mammography, future screening recommendations could be altered and more lives saved due to earlier detection of breast cancer in this population of women.

2. BODY

This section describes the research accomplishments associated with each task as outlined in the approved Statement of Work. The task has been re-stated with accomplishments to date.

Task # 1
Mail invitations for screening to the eligible population through the Florida Cancer Genetics Network (FCGN) centers. [Month #1- 6 of study]

Enrollment began on January 15, 2003. We experienced delays in beginning enrollment due to regulatory and logistical issues as reported in the October 2002 progress report. Revisions were made in response to the Memorandum for Record, Board Member's Recommendation and USF IRB recommendations. Other delays were due to the installation of a software upgrade to the MRI scanner.

Invitations were mailed to subjects known to meet the eligibility criteria from the Florida Cancer Genetics Network (FCGN). Subjects were also recruited from the Moffitt Clinical Genetics program and the internal databases of Lifetime Cancer Screening Center. A total of 129 subjects have been recruited through the end of August. Study enrollment will continue through September 30th, 2003. There are currently 58 additional subjects scheduled for study participation in the month of September.

Overall, interest in study participation has been high. Subjects who chose not to participate most often cited the following reasons: claustrophobia and the time required to participate. Subjects were told they would be here approximately 3- 3 ½ hours to complete the study procedures. If they required genetic counseling it could take longer. Many potential subjects reported claustrophobia and did not feel they could undergo the MRI.

Task # 2
Obtain informed consent, perform urine pregnancy test (if not post-menopausal or post-hysterectomy), genetic counseling (if needed), questionnaire review, and clinical breast exam (CBE). [Month # 2 – 11 of study]

Subjects were considered enrolled if they signed the informed consent. Eligibility was confirmed by the genetics counselor, who reviews the questionnaire with the subject. If not already enrolled in the Florida Cancer Genetics Network (FCGN), subjects were asked to enroll so that their confidential genetics information could be stored in the FCGN database.

All subjects who had not previously received genetic counseling for inherited breast cancer susceptibility were offered genetic counseling.

Participants who were not post-menopausal or post-hysterectomy by self-report underwent a urine pregnancy test. If the pregnancy test was positive, subjects were unable to participate.

If the clinical breast exam was abnormal, subjects were referred for standard of care, but could not participate in the study. One subject had an abnormal clinical breast exam and was referred for the routine follow-up, a full clinical diagnostic breast evaluation.

Task # 3
Perform screening MRI and mammography for participating subjects. [Month # 2 –11 of study]

Subjects with a normal CBE and a negative pregnancy test, then underwent the screening mammography and MRI procedures. Patients with implants or unilateral mastectomies were scheduled for diagnostic mammograms instead of screening mammograms, as per standard of care. If subjects have had a mammogram in the past 3 months, they did not have another mammogram, but brought their films to be interpreted for the study.

To date 129 subjects have enrolled. Ten of the subjects are off study. Four were claustrophobic and unable to complete the MRI. One was discovered to have a metallic clip and was unable to undergo MRI. One had high blood pressure, she was referred to her primary care physician for control of her hypertension and was to re-schedule her MRI when blood pressure controlled. One subject had an abnormal clinical breast exam and was referred for routine care. One subject developed lightheadedness during IV insertion and did not want to reschedule. One could not complete the MRI due to knee problems, she was going to reschedule, but is having knee surgery and is unable to reschedule before September.

One subject was discovered to be ineligible only after she had completed the exams. When her outside mammogram films were received, it was discovered that she was being followed for an abnormal finding. During screening she had indicated she was due for a screening mammogram. Our eligibility screening procedure was changed to require outside mammogram images to be available prior to the subject undergoing study procedures.

A few subjects who could not complete the MRI on the day of their mammogram due to claustrophobia, were allowed to reschedule the MRI within 3 months of the mammogram if they felt they wanted to try again.

Although subjects were questioned about claustrophobia during the screening process, often it was not discovered to be a problem until subjects were preparing to undergo the MRI. We implemented a procedure to try to prevent this problem. If subjects expressed any uneasiness with the MRI procedure, we would allow them to visit the MRI room to get an idea of what the procedure involved before agreeing to participate.

One patient developed hives following the MRI and one experienced minimal swelling and pain at the IV insertion site. Both of these patients were referred to the medical monitor for evaluation. These were determined to be anticipated events and no further intervention was required.

Mammography and MRI exams were conducted according to protocols described in the proposal.

Task # 4
Interpretation of MRI exams and mammograms [Month # 2 – 11 of study]
MRI exams and mammograms will each be interpreted independently without knowledge of the results of the other study. Each MRI will be arbitrarily assigned for interpretation to one of the study radiologists, with a different radiologist interpreting the mammogram.

All mammograms and MRIs were interpreted according to protocol. Mammographic findings were reported using the American College of Radiology Breast Imaging Reporting and Data System (BI-RADS) categories. Breast MRI studies were coded in categories equivalent to the BI-RADS classification for mammography.

Task # 5
Data entry and management [Month # 2 – 11 of study]
Data has been maintained as outlined in the protocol. The subjects' genetic information is stored in the FCGN database and is only accessible to staff associated with the genetics program. At the end of this study, subjects may choose to have their information removed from the FCGN.

Clinical data for imaging tests includes only clinically relevant demographics to comply with the federal Mammography Quality Standards Act and the imaging interpretation results. All clinical data is reported and maintained in the Lifetime Cancer Screening System reporting system. This system is accessible only to qualified personnel.

Task # 6
Clinical follow-up of screened subjects [Month # 2 – 12 of study and year 2 for follow-up only]

Enrollment on the study began January 15, 2003. Enrollment will continue through September 30, 2003. Follow-up of subjects is not complete at this time.

To date, one patient is known to have been diagnosed with breast cancer. This patient had a history of right breast cancer. Her mammogram was interpreted as BIRADS 2 and her MRI was a BIRADS 4. A follow-up ultrasound was recommended. The ultrasound BIRADS was 1. It was then recommended that she have a follow-up 3-4 month MRI. Prior to the follow-up MRI the patient developed a palpable abnormality and underwent a left ultrasound core biopsy (at an

outside facility), which revealed intraductal carcinoma. She went on to have a bilateral mastectomy. The right was benign. The left revealed invasive ductal carcinoma with a tumor size of 1.2 cm.

One subject was referred for a biopsy. Her mammogram was a BIRADS 4 and the MRI was a BIRADS 3. She was referred for an ultrasound with a result of BIRADS 4. She then underwent a biopsy which revealed a fibroadenoma. She is being followed clinically by her physician.

One subject underwent a prophylactic bilateral mastectomy, four months following the study screening exams, after her sister developed breast cancer. Her screening mammogram was a BIRADS 1 and her MRI was a BIRADS 4. A follow-up ultrasound was a BIRADS 1. The mastectomy was negative for cancer.

Task # 7
Data Analysis [Month # 12 of study and year 2 for follow-up only]

After all subjects are enrolled, imaged and their exams interpreted, the study radiologists will review and compile the image and interpretation data and the outcomes. Drs. Clark, Sutphen, Berman, and Beam will analyze the results after follow-up of one-year is completed.

At this time all subjects have not been enrolled. Enrollment will be completed September 30th, 2003.

A summary of the findings of all subjects enrolled through the end of August 2003 is provided below. To date, 129 subjects are enrolled, 10 are off study.

Table 1.

Characteristics of Subjects on Study (n = 119)

	Pre-menopausal	History of Breast Cancer	Mastectomy	Implants
No. of Subjects	49	29	9	12

Table 2.

Age distribution of Subjects on Study (n = 119)

	20-29	30-39	40-49	50-59	60-69	70-79
No. of Subjects	4	21	46	39	8	1

Of the 119 on study, 2 MRI reports and 2 mammogram reports are pending at the time of this report.

The summary of findings below is based on findings from 115 patients who have reports available for both mammograms and MRIs at the time of this report.

Table 3 demonstrates the BIRADS observed for mammogram and MRI. Overall, 71 patients had a BIRADS of 1 or 2 by both mammogram and MRI.

Table 3.

	MRI BIRADS						
Mammogram BIRADS	0	1	2	3	4	5	Total
0	3	4	-	-	-	-	7
1	17	50	1	6	2	-	76
2	6	19	1	1	2	-	29
3	-	2	-	-	-	-	2
4	-	-	-	1	-		1
5	-	-	-	-	-	-	-
Total	26	75	2	8	4	-	115

A true positive result by either imaging modality is defined as a BIRADS interpretation category of 0, 3, 4, or 5 with a subsequent diagnosis of breast cancer. A false positive result by either imaging modality is defined as a BIRADS interpretation category 0, 3, 4, or 5 without a subsequent diagnosis of breast cancer within one year.

A true negative result by either imaging modality is defined as a BIRADS interpretation category 1 or 2 without a subsequent diagnosis of breast cancer within on year. A false negative result by either imaging modality is defined as a BIRADS interpretation category 1 or 2 with a subsequent diagnosis of breast cancer within one year.

Of the 115 subjects with complete data available, 8.7 % of the mammograms and 33 % of the MRIs were interpreted as positive. The concordance observed between the MRI and mammogram interpretations is 65.2 % (see Table 4). The discordance is 34.8%.

Table 4. Concordance of Mammogram and MRI

	MRI		
Mammogram	Positive	Negative	Total
Positive	4	6	10
Negative	34	71	105
Total	38	77	115

The one-year follow-up of all patients will not be complete until September 30, 2004. To date, one patient has been diagnosed with cancer. Based on the limited follow-up available, the results are presented below.

Tables 5 and 6 show the follow-up results of a cancer diagnosis for mammography and MRI retrospectively. The overall sensitivity, specificity, PPV and NPV for mammogram and MRI are shown in Table 7. To date, as hypothesized, the sensitivity and predictive values of screening MRI are greater than that of screening mammography. The specificity for MRI is lower than that of mammography. We emphasize that the follow-up time is limited and a more thorough summary of findings will be provided at the end of the follow-up period.

Table 5. Mammogram Results

	Breast Cancer Status		
Test result	Breast Cancer	No Breast Cancer	Total
Positive	0	10	10
Negative	1	104	105
Total	1	105	115

Table 6. MRI Results

	Breast Cancer Status		
Test result	Breast Cancer	No Breast Cancer	Total
Positive	1	37	38
Negative	0	77	77
Total	1	114	115

Table 7. Sensitivity, Specificity and Predictive Value

	Mammogram	MRI
Sensitivity	0 %	100 %
Specificity	90.4 %	67.5 %
Positive Predictive Value	0 %	2.6 %
Negative Predictive Value	99 %	100 %

Task # 8
Final report and publication [Year 2]
Drs. Clark, Sutphen, Berman, and Beam will complete the final report to the funding agency in month # 12. When one-year follow-up of all subjects is complete, they will prepare a manuscript for publication in a scientific journal.

We were not given 12 months of enrollment time, due to delays in approval of the project by the DOD. Patients will be enrolled through September 30, 2003. The one-year follow-up period for the last patient enrolled would be September 30, 2004. Since we were not given 12 months of enrollment time, we could not complete enrollment of all 300 subjects. Therefore, one-year follow-up, report, and publication are not feasible.

3. KEY RESEARCH ACCOMPLISHMENTS

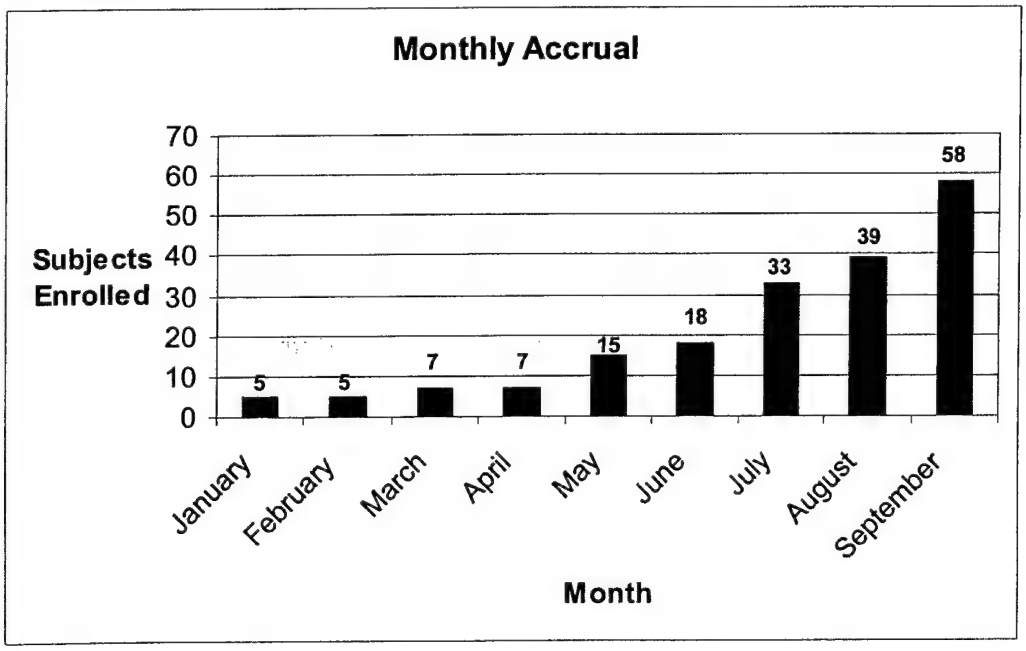
To date there are no key research accomplishments. Since we were not given 12 months of enrollment time, we could not complete enrollment of all 300 subjects. Therefore, one-year follow-up report and publication are not feasible.

4. REPORTABLE OUTCOMES

To date no reportable outcomes have resulted from this research. Since we were not given 12 months of enrollment time, we could not complete enrollment of all 300 subjects. Therefore, one-year follow-up, report, and publication are not feasible.

5. CONCLUSIONS

We originally anticipated an accrual period of one year. Our start date for enrollment was delayed due to revisions that were made in response to the Memorandum for Record, Board Members' Recommendations and USF IRB recommendations. Logistical issues have also resulted in delays. These include the establishment of the breast MRI reporting system and a software upgrade to the MRI scanner. We began enrollment on January 15, 2003. Unfortunately, since the funding period is coming to a close, our accrual period has been decreased to 9 months. Therefore, we will not meet our projected accrual of 300 patients. Our accrual has increased dramatically each month. This would be expected due to the timing of the invitations being sent out and the fact that potentially eligible subjects are scheduled at the time that they are due for their routine screening. Also, as the study progresses more potential subjects become aware of the study. Since we were not given 12 months of enrollment time, we could not complete enrollment of all 300 subjects. Therefore, one-year follow-up, report, and publication are not feasible.



At the current accrual rate we believe we could easily have met our accrual goal of 300 by January of 2004. Interest in study participation has been high.

We will not be able to draw any conclusion regarding the outcome until all patients have been enrolled and follow-up is complete. Our information does suggest that a future expanded trial would be successful and would warrant additional funding. Because the ideal screening recommendations for women at a high-risk for breast cancer is currently uncertain, further investigation of breast MRI screening is justified.

Appendix I.

Key personnel who have received salary support on this project:

Principal Investigators:
Robert A. Clark, M.D.
A. Pat Romilly, M.D.

Co-Investigators:
Rebecca Sutphen, M.D.
Claudia Berman, M.D.
Craig Beam, Ph.D.

Genetic Counselors:
Jenny Permuth-Wey
Jennifer Pickard
Judith Betts

Research Nurse:
Elissa Clayton
Lisa Roberts

Data Base Manager:
Jamie Malloy
Veena Gowda

Clinical Research Assistants:
Joann Runk
Kim Jefferson
Dan Shippy
Rosaline Drew

APPENDIX E

The Tampa Bay Ovarian Cancer Study

Rebecca Sutphen, M.D.

Tampa Bay Ovarian Cancer Study
Principal Investigator: Rebecca Sutphen, M.D.

1. Introduction

The BRCA1 and BRCA2 genes are believed to account for the majority of inherited ovarian cancer, yet few population-based studies have been performed specifically to investigate their role in this deadly disease – there are no population-based reports from the U.S. The largest population-based study to date was done in Ontario, Canada*, based on 649 unselected cases of ovarian cancer, of which 515 were invasive. Methodology for mutation detection included screening of exon 11 of BRCA1 and exons 10 and 11 of BRCA2 through protein truncation test. In addition, all samples were screened for 11 common mutations by rapid multiplex method (including 3 Jewish founder mutations and 6 French Canadian mutations). Utilizing this limited mutation detection strategy, among the 515 women with invasive ovarian cancer, 60 mutations (11.7%; 95% Confidence Interval: 9.2-14.8%) were identified, among which 39 mutations were identified in BRCA1 and 21 mutations were identified in BRCA2. The average age at diagnosis of BRCA1 carriers, BRCA2 carriers, and sporadic cases was 51.2, 57.5, and 56.7 years, respectively. Of the 60 mutation carriers, 38 patients (63%) had a positive family history of breast and/or ovarian cancer, and 22 patients (37%) did not. Pathologic analysis showed that 56 of the 60 (93%) mutation carriers had invasive serous cancers, and the remaining 4 women had endometrioid tumors.

The Tampa Bay Ovarian Cancer Study (TBOCS) established a coalition of investigators to perform a population-based case-case study of incident epithelial ovarian cancer in a heavily populated 2-county region of west central Florida. This coalition, including regional community physicians, was used to accrue incident cases of ovarian cancer diagnosed between December 13, 2000 and September, 30, 2003, through a rapid case ascertainment mechanism in Hillsborough and Pinellas counties, Florida, including the greater Tampa - St. Petersburg - Clearwater metropolitan area with a population in excess of 2 million. Through this study, 231 women diagnosed with ovarian cancer between the ages of 18-80 were enrolled. In-person interviews were conducted with all subjects, in order to collect comprehensive data on health behaviors, risk factors, personal and family history; provide genetic counseling; and obtain blood samples. Complete sequencing of the BRCA1 and BRCA2 coding regions was performed to allow assignment of cases (mutation-carriers) and controls (non-carriers) and determination of the prevalence of BRCA1 and BRCA2 germline mutations in this population.

The aims of this study of incident epithelial ovarian cancer are:

1) to investigate whether and which health behaviors and risk factors differ between germline mutation-associated cases and non-mutation controls;

2) to examine differences in the family cancer history profile of mutation-associated cases and non-mutation controls;

3) to examine differences in tumor characteristics between mutation-associated cases and non-mutation controls;

4) to investigate differences in response to treatment and survival between mutation-associated cases and non-mutation controls;

5) to achieve an 80% participation rate.

2. Body

The study was reviewed by the Surgeon General's Human Subjects Research Review Board (SGHSRRB) on September 27, 2000. Final approval to open the study for enrollment was obtained on December 13, 2000.

*Risch, HA, McLaughlin, JR, Cole, DEC et al. Prevalence and Penetrance of Germline BRCA1 and BRCA2 Mutations in a Population Series of 649 Women with Ovarian Cancer. Am.J.Hum.Genet. 68: 700-710, 2001.

Data Compilation of currently available data on 231 participants enrolled in the study is summarized in table 1 below:

Table 1

Information	Number Available	Number Pending
All TBOCS paticipants (n=231)		
BRCA1 and BRCA2 mutational analysis	210	21
Pathology Report	171	60
Tumor Blocks Collected	150	81
Ethnicity Data	189	42
Family History Information	173	58
Menopausal Status	138	93
Mutation Carriers (n=31)		
Pathology Report	29	2
ER/PR Status	22	9
Menopausal Status	13	18

Status of tasks included in the approved statement of work is as follows:

Task 1: Preparation for Medical Record Abstractions

Data elements of the medical record abstraction form were finalized. Design of the medical record abstraction form and the data entry mechanism for pathology data were completed. This design allows direct data entry of abstracted medical records into the database, followed by independent review of the medical record data by the pathologist at the time of pathology analysis. This mechanism made data entry highly efficient while ensuring data quality.

Task 2: Recruitment and Training of Study Personnel

We enrolled 231 ovarian cancer patients in this study through their treating physician. Data from the Florida Cancer Datasystem shows that in women diagnosed with invasive epithelial ovarian cancer between age 18-80, ~85% of regional cases are diagnosed by 7 gynecologic oncologists, hence recruitment strategies involved recruiting all gynecologic oncologists in the region as co-Investigators and training their staff regarding the study. This first strategy was accomplished, except that one of the 7 gynecologic oncologists refused participation, despite several strategies to facilitate participation, hence only a limited number of his patients could be enrolled in this study. Through several strategies used to centralize responsibility for patient contact to study staff, we were able to decrease responsibilities of local physicians' staff, which facilitated enrollment.

Task 3: Subject recruitment and Data Collection

Patient recruitment for this study involved the treating gynecologic oncologist introducing the TBOCS study to their patients with incident invasive epithelial ovarian cancer, and if patients were agreeable to study participation, the study team was informed. A genetic counselor scheduled an in-person meeting with the patient at the gynecologic oncologist's office, or at another convenient location for the patient. The study interview was a successful strategy to accomplish explanation of the study, provision of informed consent, enrollment, completion of study questionnaire, genetic counseling, and blood sampling. Medical records have been successfully obtained for all recruits on whom they have been requested, however there are outstanding medical records on 60 recently recruited women, and these records are in the process of being obtained. Tumor tissue has been successfully obtained from the appropriate surgical locations for pathology analyses on 150 patients thus far. Tumor blocks are in the process of being collected on the remaining, more recently recruited, TBOCS participants. Paraffin embedded tumor block specimens have been successfully obtained on every patient on whom they were requested, and all remaining samples on the recently recruited women will be collected within the next few months. Blood samples

have been successfully obtained for genetic testing and banking for future research on all women recruited into the study.

Data on the initial 113 patients enrolled in the study has been extracted and show a distribution of histological subtypes, stage, and median age of diagnosis similar to that seen in the general population (refer to table 2):

Table 2: Description of the data from the first 113 TBOCS patients.

	Expected number based on general population	Actual number seen in TBOCS study (n=113)
Histology:		
Serous	75%	72% (81/113)
Endometrioid	15-25%	16% (18/113)
Stage Distribution:		
Stage I/II	30%	26% (29/113)
Stage III/IV	70%	74% (84/113)
Median Age:	63	59

The two most populous counties within the Moffitt catchment area are Hillsborough and Pinellas, which account for ~75% of the population, and were the counties from which TBOCS participants were recruited. Data has been compiled on the initial 189 TBOCS patients, and shows that 55% are from Hillsborough and 45% are from Pinellas. Table 3 summarizes the catchment area demographics, the distribution of cancer cases for this area and the comparable distribution of cases seen through TBOCS. Demographic data are culled from the 1990 census; incident cancer case data for the catchment area is from the State Cancer Registry 1998 data. The distribution of TBOCS patients by race/ethnicity is similar to the distribution of cancer cases in the catchment area, as shown in the table below (Table 3).

Table 3. Ethnic Background of TBOCS patients compared to demographics Tampa Bay Region

Race /Ethnicity	Catchment Area Demographics	Catchment Area Cancer Cases	TBOCS patients (N=189)
White/Not Hispanic	84%	91%	89.5%
Hispanic	5.9%	2.8%	6.4%
Black/Not of Hispanic origin	9%	4.7%	1.6%
American Indian	0.2%	0.0%	1.6%
Asian/Pacific Islander	0.9%	0.3%	0.5%
Other		0.0%	0.5%
Unknowns		1.1%	

Task 4: Disclosure of results to the patients

The results of genetic testing and related information (depending on results) were provided to subjects who elected to receive results. Of the 231 women recruited, only 1 woman elected not to receive results. Results of genetic testing were utilized for assignment of case-control status and matching. Results are currently available on 210 of these women. Of the first 210 women enrolled in the study, 31 (14.8%) had mutations in BRCA1 or BRCA2: 20 in BRCA1 (9.5% of cases) and 11 in BRCA2 (5.2%).

The mutations in the BRCA1 gene were distributed throughout the 5 regions of the gene, as shown in Table 4. Of the BRCA2 mutations, 45% (5/11) were outside the Ovarian Cancer Cluster Region (OCCR), as shown in Table 5. The higher rate of mutation detection compared to the Ontario study was, in part, due to comprehensive analysis performed in this study.

Table 4: BRCA 1 mutations (n=20)

Specific BRCA1 Mutation	Region of BRCA1
2530delAG	3
C944X	3
2576delC	3
187delAG	1
3790ins4	4
5385insC	5
187delAG	1
4154delA	4
4440insG	4
E1134X	3
3875del4	4
187delAG	1
K679X	2
2800delAA	3
187delAG	1
187delAG	1
C61G	1
2800delAA	3
2576delC	3
1294del40	2

Table 5: BRCA2 mutations (n=11)

Specific BRCA2 Mutation	OCCR of BRCA2
Q1931X	yes
Q2009X	yes
2041insA	no
R2520X	no
6174delT	yes
4512insT	yes
1983del5	no
E49X	no
6174delT	yes
R2520X	no
4706del4	yes

The percentages of patients having a positive family history of breast and/or ovarian cancer in a first or second degree relative for BRCA1 carriers (n=20), BRCA2 carriers (n=11), and women with sporadic ovarian cancer (n=142) were 65%, 82% and 30%, respectively. The average ages of diagnosis for these 3 groups of women were 53, 58, and 59, respectively. Personal and family history information on cancer diagnosis has been compiled on 31 mutation carriers and 142 sporadic cancers and is shown in table 6 below:

Table 6: Personal and family history of cancer in participants

Family History	BRCA1 (n=20) 65%	BRCA2 (n= 11) 35%	BRCA+ (n= 31)	Sporadic (n=142)
Negative family history of breast or ovarian cancer in 1st or 2nd degree relative	n=7 (35%)	n=2 (18%)	n=9 (29%)	99 (70%)
Positive family history of breast or ovarian cancer in 1st or 2nd degree relative	n=13 (65%)	n=9 (82%)	n=22 (71%)	43 (30%)
Personal history of breast and ovarian cancer	n=6 (30%)	n=3 (27%)	n=9 (29%)	0 (0%)
Age at diagnosis of ovarian cancer				
≤40	0 (0%)	1 (9%)	n=1 (3%)	15 (10.6%)
41-50	8 (40%)	2 (18%)	n=10 (32%)	21 (14.8%)
51-60	10 (50%)	2 (18%)	n=12 (39%)	25 (17.6%)
>60	2 (10%)	6 (55%)	n=8 (26%)	39 (27.5%)
Range	42-77	34-73	34-77	33-80
Mean	53	58	54	59

This data compares to the limited population-based data available from published reports, showing that family history may not predict germline BRCA1 and BRCA2 mutations in a substantial proportion of carriers. Additionally, the mean age of onset of ovarian cancer in BRCA1 carriers is reported to be about 5 years earlier than that seen BRCA2 carriers. In BRCA2 carriers, mean age at diagnosis is similar to that seen in the general population, consistent with previous reports.

Task 5: Abstraction of Medical Records

Of the 231 women in this study, medical record abstractions have been completed for 171 enrolled subjects (ie: 142 sporadic cancers and 29 mutation carriers) and 6 month, 12 month and 24 month follow-up data has been obtained for appropriate participants. Data abstracted from medical records includes review of histologic subtype, grade, and stage of cancer and is shown in the tables below (table 7 and table 8).

Table 7: Data from medical record review of carriers

Histology	BRCA1 (n=18) *	BRCA2 (n=11)	BRCA+ (n=29)*	Sporadic (n=142)
Serous	12 (67%)	5 (45.5%)	17 (58.6%)	62 (43.7%)
Endometrioid	1 (5.5%)	2 (18.2%)	3 (10.3%)	15 (10.6%)
Transitional	1 (5.5%)	0 (0%)	1 (3.4%)	6 (4.2%)
Mucinous	0 (0%)	0 (0%)	0 (0%)	5 (3.5%)
Mixed	4 (22%)	1 (9.1%)	5 (17.2%)	8 (5.6%)
Peritoneal	0 (0%)	1 (9.1%)	1 (3.4%)	0 (0%)
Brenner	0 (0%)	1 (9.1%)	1 (3.4%)	0 (0%)
Unknown	0 (0%)	1 (9.1%)	1 (3.4%)	0 (0%)

* data is not available on 2 of the 20 BRCA1+ women

As seen in table 7, most of the tumors had serous histology, and none were mucinous or borderline tumors, similar to previous literature reports. In addition, of the 22 mutation carriers on whom this data has been compiled, all BRCA1- and BRCA2- associated ovarian cancers were ER-/PR- and ER+/PR+ respectively, consistent with previous reports.

Table 8: Stage and Grade Information in BRCA1 and BRCA2 carriers

	BRCA1 (n=18) *	BRCA2 (n=11)	BRCA+ (n=29)*
Stage			
I	1 (6%)	4 (36.4%)	5 (17.2%)
II	2 (11%)	1 (9.1%)	3 (10.3%)
III	13 (72%)	5 (45.5%)	18 (62.1%)
IV	2 (11%)	1 (9.1%)	3 (10.3%)
Grade			
1	4 (22%)	3 (27.3%)	7 (24.1%)
2	2 (11%)	1 (9.1%)	3 (10.3%)
3	12 (67%)	7 (63.6%)	19 (65.5%)

* data is not available on 2 of the 20 BRCA1+ women

As seen in table 8, the distribution by stage of BRCA carriers was similar to that seen in the general population, where ~15% present in stage 1 and 70% present in stages 3 or 4. However, 17% (3/18) of BRCA1 carriers compared to 45.5% (5/11) of BRCA2 carriers presented with early stage disease (ie: stage 1 or 2), with a p-value of 0.08, based on the Fisher's Exact Test. We suspect that this borderline significant value is due to the small number (ie: 3) of observed early stage BRCA1 carriers.

Task 6: Tumor Tissue Analyses

Tumor tissue has been collected for the first 150 recruits thus far, and is in the process of being collected for the remainder. Pathology analyses are being completed and tissue will then be banked for future research.

Task 7: Follow-up for Survival

Follow-up data at 6 months, 12 months and 24 months has been obtained as appropriate. Follow-up contacts will continue for surviving enrolled subjects after 6 months, after 1 year, and annually as funding permits. Data entry and quality control measures are ongoing. Data on the initial 100 women recruited into the study indicates that 15 women have died thus far.

Task 8: Statistical Analyses and report writing

Interim analyses include acceptance for publication in *Cancer Epidemiology, Biomarkers, and Prevention*, of the data arising from the lysophospholipids screening study (Appendix A). Interim analyses of the first 100 women enrolled in TBOCS were presented at the 2002 American Society of Human Genetics annual meeting (Appendix B). Interim analyses of the first 164 women enrolled in TBOCS has been accepted for presentation at the 2003 Frontiers in Cancer Prevention Research annual meeting of the American Association for Cancer Research (Appendix C). Final analyses and reports are in the process of being prepared, pending final data collection.

3. Key Research Accomplishments

Aim 1: Data regarding health behaviors and risk factors were obtained from all participants via questionnaire instruments and study interview was successfully completed on all 231 women. Data regarding menopausal status has been compiled on 138 women. Of the 13 mutation carriers on whom data has been compiled, 0, 4, and 9 are pre-, peri-, and post-menopausal, respectively. Of the 125 sporadic cases on whom data has been compiled, 17, 26, and 82 are pre-, peri-, and post-menopausal, respectively.

Aim 2: Detailed cancer family history was obtained from all participants via questionnaire instruments and study interview.

Aim 3: A successful mechanism has been implemented to obtain medical records and tumor tissue in order to compare tumor characteristics between mutation-associated cases and non-mutation controls.

Aim 4: A successful follow-up mechanism has been implemented to obtain data regarding differences in response to treatment and survival between mutation-associated cases and non-mutation controls.

Aim 5: Although we were not able to achieve an 80% participation rate, we have been able to accrue a population-based sample with regard to ethnicity, stage, histologic subtype, median age and ethnicity (refer to Tables 2 and 3). Based on Florida Cancer Data System (FCDS) data, we estimate that a total of 430 patients were diagnosed with ovarian cancer during the study period, of whom 350 patients were ascertained (ie: 82%). There were 12 patients who died prior to enrollment, 18 patients or doctors declined, and a further 10 patients who could not be enrolled prior to the closing of the study. Hence of the 350 cases ascertained, 231 were enrolled in the study (66%).

4. Reportable Outcomes

Based on the epidemiologic design of the Tampa Bay Ovarian Cancer Study, funding was awarded by the American Cancer Society for a companion study to evaluate the role of biologically active lysophospholipids for their potential as biomarkers of ovarian cancer (7/1/00 – 6/30/04). Preliminary data is promising and shows that certain lysophospholipids appear to be elevated in the plasma of women with ovarian cancer compared with healthy controls (article accepted in Cancer Epidemiology, Biomarkers, and Prevention and included in Appendix A). We have applied to ACS for a two-year extension of the project. Also, based on this preliminary data, we have applied to NIH for R01 funding to investigate the use of lysophospholipid measurement and proteomic profiles for detection of ovarian cancer in a case-control study.

Based on data showing that gene mutations associated with Hereditary Non-Polyposis Colorectal Cancer (HNPCC) are the third leading cause of hereditary ovarian cancer (after BRCA1 and BRCA2), and the suggestion that ovarian cancer is a "sentinel cancer" in individuals with these gene mutations, an investigation of HNPCC as a companion study of TBOCS has been funded.

The preliminary results of this research were presented at the 2002 American Society of Human Genetics annual meeting (Appendix B), and 2003 Frontiers in Cancer Prevention Research (American Association for Cancer Research) annual meeting (Appendix C).

5. Conclusions

Epithelial ovarian cancer results in the death of more American women than all other gynecologic cancers combined. The Tampa Bay Ovarian Cancer Study was the first U.S. population-based study to evaluate the role of the BRCA1 and BRCA2 genes in the etiology, pathology and response to treatment of this deadly disease. Findings from this study include: 1) The incidence of hereditary ovarian cancer due to BRCA1 and BRCA2 mutations is higher than previously reported; 2) BRCA2 mutations account for a higher percentage of hereditary ovarian cancer cases than previously reported; 3) Previous studies may have underestimated the contribution of BRCA2 to ovarian cancer, especially mutations outside the ovarian cancer cluster region (OCCR); 4) approximately 30% of hereditary ovarian cancer patients have no obvious family history to suggest hereditary cancer susceptibility, hence family history may not be sufficient to accurately predict mutations. A companion study to assess the potential of biologically active lysophospholipids as biomarkers in ovarian cancer was funded and is in progress. The Tampa Bay Ovarian Cancer Study and its companion study represent an important opportunity to evaluate the role of inherited susceptibility to ovarian cancer and evaluate lysophospholipids for their potential as biomarkers of this deadly disease.

APPENDIX A

Lysophospholipids Are Potential Biomarkers of Ovarian Cancer

Rebecca Sutphen, M.D.[1], Yan Xu, Ph.D.[2], George D. Wilbanks, M.D.[3], James Fiorica, M.D.[1,3], Edward C. Grendys, Jr., M.D.[1,3], James P. LaPolla, M.D.[4], Hector Arango, M.D.[5], Mitchell S. Hoffman, M.D.[6], Martin Martino, M.D.[3], Katie Wakeley, M.D.[3,8], David Griffin, M.D.[6], Rafael W. Blanco, M.D.[8], Alan B. Cantor, Ph.D.[1], Yi-jin Xiao[2], Ph.D., and Jeffrey P. Krischer, Ph.D.[1]

1. Department of Interdisciplinary Oncology, University of South Florida College of Medicine, H. Lee Moffitt Cancer Center & Research Institute, Tampa, FL
2. Cleveland Clinic Foundation, Cleveland, OH
3. Department of OB/GYN, University of South Florida College of Medicine, Tampa, FL
4. Department of GYN/Oncology, Bayfront Medical Center, St. Petersburg, FL
5. Morton Plant Hospital, Clearwater, FL
6. Department of GYN/Oncology, University of South Florida, Tampa, FL
7. Tufts University, New England Medical Center, Boston, MA
8. Bay Area Oncology, Tampa, FL

Corresponding Author

Rebecca Sutphen, M.D.

H. Lee Moffitt Cancer Center & Research Institute

12902 Magnolia Drive, FOW-LCS

Tampa, FL 33612

(813) 903-4990/(813) 558-4807 fax

rsutphen@hsc.usf.edu

Lysophospholipids Are Biomarkers of Ovarian Cancer

Abstract

Objective

To determine whether lysophosphatidic acid (LPA) and other lysophospholipids (LPL) are useful markers for diagnosis and/or prognosis of ovarian cancer in a controlled setting.

Method

Plasma samples were collected from ovarian cancer patients and healthy control women in Hillsborough and Pinellas counties, Florida, and processed at the H. Lee Moffitt Cancer Center and Research Institute at the University of South Florida (Moffitt). 117 case patients with epithelial ovarian cancer and 27 healthy control subjects participated in the study. Blinded LPL analysis, including 23 individual LPL species, was performed at the Cleveland Clinic Foundation, using an electrospray ionization mass spectrometry-based method. LPL levels were transmitted to Moffitt where clinical data were reviewed and statistical analyses performed.

Results

There were statistically significant differences between preoperative case samples (N=45) and control samples (N=27) in the mean levels of total LPA, total lysophosphatidylinositol (LPI), sphingosine-1-phosphate (S1P) and individual LPA species, as well as the combination of a number of LPL species. The combination of 16:0-LPA and 20:4-LPA yielded the best discrimination between preoperative case samples and control samples, with 93.1% correct classification, 91.1% sensitivity and 96.3% specificity. In 22 cases with both preoperative and postoperative samples, the postoperative levels of a number of LPL, including S1P, total LPA and LPC levels and some individual species of LPA and LPC, were significantly different from preoperative levels.

Conclusion

LPA, LPI, LPC and S1P appear useful as diagnostic and prognostic biomarkers of ovarian cancer.

Introduction

The mortality rate for women with ovarian cancer is very high, with an estimated 14,300 deaths from ovarian cancer in 2003 in the United States.[1] More than two-thirds of patients have late stage metastatic disease at initial diagnosis with a 5-year survival rate of approximately 20 - 30%.[1-4] Conversely, at early stages, the long-term survival rate approaches 90%.[5] There is currently no proven effective method for early detection of ovarian cancer through biomarkers, imaging, or other means. The most common biomarker for ovarian cancer, CA 125, lacks specificity and is elevated in only about 50% of stage I ovarian cancer cases.[3,4,6] Proteomic patterns derived from surface-enhanced laser desorption/ionization mass spectroscopy analysis have recently shown promise for early ovarian cancer detection[7] but further studies regarding their reproducibility and reliability for early detection and screening are needed.

Lysophosphatidic acid (LPA) has been proposed as a sensitive biomarker.[8] However, studies investigating the utility of LPA as a biomarker for early detection of ovarian cancer have yielded conflicting results. Preliminary findings from a study which included 48 healthy controls and 48 women with ovarian cancer showed that plasma LPA levels (measured by gas chromatography) were elevated in patients with ovarian cancer ($P<0.001$).[8] Importantly, elevated levels were detected in early-stage ovarian cancers compared with controls.[8] The study also compared available CA125 values with LPA levels and results suggested that plasma LPA may be a more sensitive marker for ovarian cancer, particularly for stage I disease.[8] A recent Korean study of only 3 pairs of samples also showed differences between ovarian cancer cases and controls.[9] However, in another study where LPA levels were measured in plasma samples from 32 patients with ovarian cancer and 32 healthy controls using a liquid chromatography / mass spectroscopy assay, results showed no significant elevation in plasma LPA levels in

ovarian cancer patients compared to controls, raising questions about the utility of plasma LPA levels for early detection of ovarian cancer.[10]

LPA is present in the ascitic fluid of patients with ovarian cancer[11, 12] and may function as an autocrine factor, contributing to ovarian cancer proliferation, cell survival, angiogenesis and metastasis.[13-22] Lysophosphatidylinositol (LPI), a related LPL to LPA, has also been found at increased levels in ascites fluid and plasma of ovarian cancer patients compared with controls[23] and has been shown to display signaling properties in cellular systems.[24, 25] Thus, LPI may also have utility as a biomarker of ovarian cancer, and data suggests that measuring LPI in addition to LPA may increase the sensitivity and/or specificity of the test.[23] Both LPA and LPI represent various subspecies with different fatty acid chains. In addition, the fatty acid chain may link to the glycerol backbone through different chemical linkages resulting in various subclasses (i.e., acyl- (LPA), alkyl- (A-LPA), and alkenyl- (An-LPA). Findings of a study to evaluate the discriminating ability of LPA and LPI *subspecies* for ovarian cancer identification compared with *total* LPA and LPI suggested that subspecies with unsaturated fatty acid chains may be associated with late-stage or recurrent ovarian cancer.[26] Other LPL that have been proposed to have a biologic role in ovarian cancer and be potentially useful as biomarkers of the disease include lysophosphatidylcholine (LPC), which has also been shown to be elevated in the plasma of ovarian cancer patients[27], and the lysosphingolipid sphingosine-1-phosphate (S1P) which is known to have both extracellular and intracellular signaling properties.[28-31]

To further explore the potential of LPA, LPI, LPC and S1P as biomarkers for ovarian cancer detection, we measured plasma LPL levels (including subspecies of LPA, LPI and LPC) in women with ovarian cancer and healthy controls, using an electrospray ionization mass spectrometry (ESI/MS) method recently developed by Dr. Xu's group at the Cleveland Clinic

Foundation.[23] This assay allows simultaneous detection and quantitation of different species of LPL with at least 10 times more sensitivity than the previous gas chromatography method.[23]

Materials and Methods

Patients

All patient-derived biologic specimens were collected under protocols approved by the Institutional Review Board of the University of South Florida and all participants provided written informed consent.

Whole blood samples were obtained preoperatively in EDTA tubes by routine venipuncture of women undergoing surgery for suspected ovarian cancer in Hillsborough and Pinellas counties, Florida between December 13, 2000 and October 30, 2002. All women ages 18 – 80 undergoing surgery for suspected ovarian cancer in the two counties during the defined period were regarded as eligible for entry into the study. No patients who were asked refused to participate. Of the preoperative samples obtained, 45 were from women who were later confirmed to have ovarian cancer or primary peritoneal cancer (ovarian cancer patients) (median age 60 years, range 33-79). Samples were obtained postoperatively from ovarian cancer patients from the same eligibility pool (N=94, median age 59, range 26-80), including 22 patients who had contributed a preoperative sample and 72 who had not. Whole blood samples from control subjects were collected concurrently from healthy women from the same counties who reported no history of cancer, gynecologic disease, oophorectomy or family history of breast/ovarian cancer (N= 27, median age 45, range 22-79). Whole blood specimens were obtained from a total of 117 ovarian cancer patients, including 18 patients with stage I disease, 11 with stage II disease, 74 with stage III disease and 14 with stage IV disease. Among the 45 patients for whom a preoperative sample was available, there were 7 patients with Stage I disease, 3 with Stage II disease, 31 with stage III disease and 4 with stage IV disease. Cancer diagnosis was confirmed for all cases by review of pathology records by a single ovarian cancer expert. Clinical stage was

determined according to International Federation of Gynecologists and Obstetricians criteria[32], and the histologic subtype was evaluated according to the World Health Organization classification.[33]

Sample Collection

LPA is produced and released by activated platelets during coagulation and therefore is a normal constituent of serum, but it is present only at very low levels in whole blood or fresh platelet-poor plasma from healthy individuals.[8] To prevent platelet activation and phospholipase activity, blood samples were collected in EDTA-containing tubes. Since LPLs are metabolites and levels may change during incubation, it is important that sample processing be as consistent as possible across all samples for comparison. We collected samples from multiple locations in the two study counties and processed (centrifugation and aliquotting) all samples at the Moffitt Cancer Center. After blood drawing, samples were immediately chilled for transport to Moffitt by being placed in a styrofoam container accompanied by a frozen pack for overnight delivery. This system allowed centrifugation within 16-28 hours after blood drawing. Centrifugation was at 3000g for 20 minutes after which the plasma was immediately aliquotted per each 0.5 cc into coated microEppendorf tubes and immediately frozen at –70°C. Samples were batch-shipped on dry ice by overnight delivery to the Cleveland Clinic for analysis. Shipped samples were identified by a unique sample number only, without identifiers or any indication of the subject's status as ovarian cancer patient or control. The samples were maintained at –70°C until preparation for mass spectrometry analysis. No personnel at the Cleveland Clinic had knowledge of the subjects' disease status at any time. Laboratory data was transmitted according to each unique sample number to the Moffitt Cancer Center where all statistical analyses were performed.

LPL Analysis

Lipids were extracted as described previously with minor modifications.[23, 34] To 0.5 mL plasma, 2 mL of MeOH/chloroform (2:1) and 0.1 mL of 6 N HCl were added. Samples were vortexed for 1 min and incubated on ice for 10 min. 1 mL of chloroform and 1 mL of H2O were added to separate the phases. Samples were vortexed for 0.5 min prior to centrifugation (2,000 g for 10 min). The lower phase was transferred to a new glass tube. To the upper phase left in the original tube, 1 mL of chloroform was added to extract more lipids and the tube was centrifuged (2,000 g for 10 min). The lower phase was transferred into the same tube (with the lower phase extract) and the solvent was evaporated under nitrogen at 30°C. The dried lipids were suspended in 50 μL of solvent (MeOH:chloroform, 2:1), vortexed, and applied to a thin-layer chromatography (TLC) plate. Two standards (18:1-LPA and 18:1-LPC) were applied to help in identifying the "LPA band" and the "LPC band" on each TLC plate. The TLC plates were developed in the solvent system (chloroform:MeOH:AmOH, 65:35:5.5) until the solvent front was 1.5 inch from the top of the plate. The lipids from the "LPA band" and the "LPC band" were eluted with 2 mL of MeOH:chloroform (2:1) twice. The lipid solutions were dried under nitrogen at 30°C and lipids resuspended in 100 μL of MeOH for mass spectrometry.

Mass spectrometry analyses were performed using a Quattro Ultima triple quadrupole ESI-MS (Micromass, Inc., Beverley, MA) with the Masslynx data acquisition system. A Waters 2690 (Waters) autosampler was used to introduce the samples into the ESI source. The mobile phase used for all experiments was MeOH:H2O (9:1; v:v) and the flow rate was 100 μL/min. The injection volume was set to 20 μL/sample for all experiments. The positive or negative ion-mode with multiple reaction monitoring (MRM) was used to quantitatively analyze the positively or negatively charged phospholipids. The collision energies were 70 eV in the negative mode and 25 eV in the positive mode. Nitrogen was used as both drying and nebulising gas at flow

rates of 500 L/h and 50 L/h, respectively. The ESI probe capillary was held at 3 kV for the positive mode and *3 kV for the negative mode and the cone voltage was set at 35V in positive mode and *50 V in negative mode. The source and desolvation temperatures were 100°C and 200°C, respectively.

LPA and other negatively charged LPL were analyzed in the negative mode with the monitoring ions at m/z 378 (parent ion) - 79 (product ion) for S1P, 381-79 for 14:0-LPA, 393-79 for 16:0- Alkenyl- LPA, 395-79 for 16:0 -Alkyl- LPA, 409-79 for 16:0-LPA, 421-79 for 18:0-Alkenyl-LPA, 423-79 for 18:0-Alkyl-LPA, 433-79 for 18:2-LPA, 435-79 for 18:1-LPA, 437-79 for 18:0-LPA, 571-79 for 16:0-LPI, 599-79 for 18:0-LPI, and 619-79 for 20:4-LPI, respectively. All lipids with the phosphorylcholine group (positively charged) were analyzed in the positive mode. Monitoring ions were at m/z 465 (parent ion) - 184 (product ion) for SPC, 496-184 for 16:0-LPC, 510-184 for 17:0-LPC, 520-184 for 18:2-LPC, 524-184 for 18:0-LPC, 544-184 for 20:4-LPC and 568-184 for 22:6-LPC, respectively. The dwell time in the MRM mode was 0.11 ms and the scan delay was 0.02s.

Statistical analysis

Categorical variables were analyzed using Chi-square tests or Fisher's exact tests, depending on sample size. Continuous variables, including univariate comparisons for quantitative variables between normal and cancer cases, were compared using the Student's t-tests, or the Wilcoxon Rank Sum test, depending on the distribution of the variable of interest. Adjustment for potential confounding variables, such as the stage at diagnosis, was carried out by using general linear modeling or analysis of variance methods, as appropriate. Stepwise logistic regression analysis was used to determine the statistical significance of LPA, LPI, LPC (and their subspecies) and S1P. All statistical significance testing was 2-sided, and *P* values less than .05 were considered to be statistically significant. *P* values in the range of .01 to .05 should

be interpreted with caution because of multiple testing issues. Statistical analyses were performed utilizing SAS Software, SAS Institute Inc, Cary, NC.

Results

The ages, stages, grades, histologic subtypes and treatment status of the 117 ovarian cancer patients who participated in the study are shown in Table 1. A total of 166 samples were analyzed, including 27 from healthy controls, 45 obtained preoperatively from women with ovarian cancer and 94 obtained postoperatively from women with ovarian cancer, with 22 patients having both preoperative and postoperative samples.

There were statistically significant differences between preoperative case samples (N=45) and control samples (N=27) in the mean levels of several individual LPA species, the combination of 16:0-LPA/20:4-LPA, total LPA, total LPI and S1P (Table 2). The best discrimination between samples obtained preoperatively from ovarian cancer patients and those from healthy controls was achieved by the combined levels of 16:0-LPA and 20:4-LPA , with 93.1% correct classification, 91.1% sensitivity and 96.3% specificity (Figure 1). Receiver operating characteristic curves (ROC)[35] were examined and a cutoff 16:0-/24:0-LPA level of 0.62 μM was identified as optimizing the sensitivity and specificity of the assay (Figure 1). All patients with preoperative samples had 16:0-/24:0-LPA levels above the 0.62 μM cutoff, with the exception of one stage I patient, one stage II patient and 2 stage III patients. Using an ROC-derived cutoff value of 1.5 μM, total LPA levels achieved 91.7% correct classification, 91.1% sensitivity and 92.6% specificity (Figure 2). All 4 of the cases which had 16:0-/20:4-LPA levels below the 0.62 μM cutoff also had low total LPA levels, as might be expected since total LPA includes 16:0-LPA and 20:4-LPA. Similarly, the control with an elevated 16:0-/20:4-LPA level of 0.91 μM also had the highest total LPA level.

The mean values for the combination of 16:0-LPA/20:4-LPA in the plasma samples obtained preoperatively from patients with stage I, stage II, stage III and stage IV ovarian cancer were 1.23 μM (S.D. 0.52), 0.92 μM (S.D. 0.43), 1.23 μM (S.D. 0.70) and 0.93 μM (S.D. 0.15), respectively, compared with 0.35 μM (S.D. 0.17) for the controls (Table 2). The mean values of total LPA in the plasma samples obtained preoperatively from patients with stage I (7 patients), stage II (3 patients), stage III (31 patients) and stage IV (4 patients) ovarian cancer were 2.57 μM (S.D. 0.94), 2.15 μM (S.D. 0.71), 2.93 μM (S.D. 1.77) and 1.97 μM (S.D. 0.27) μM, respectively, compared with 0.90 μM (S.D. 0.43) for 27 healthy controls (Table 2). The mean values of total LPI in the plasma samples obtained preoperatively from patients with stage I, stage II, stage III and stage IV ovarian cancer were 2.98 μM (S.D. 1.57), 4.58 μM (S.D. 2.71), 4.25 μM (S.D. 2.81) and 2.96 μM (S.D. 0.33), respectively, compared with 1.51 μM (S.D. 0.79) for the controls (Table 2).

In 22 cases with both preoperative and postoperative samples, the postoperative levels of total LPA, total LPC, 22:6-LPA, 18:0-LPA, the combination of 20:4-LPA/22:6-LPA, 20:4-LPC and 18:2-LPC were significantly lower than preoperative levels (P= .03, .05, .02, .04, .03 .02, .003 and .03, respectively) (Table 3). Of these LPL, 18:0 LPC, 18:2 LPC and total LPC levels also showed statistically significant differences between preoperative case samples (N=45) and all postoperative case samples (N=94) ($P \leq .05$).

Discussion

Ovarian cancer is a disease associated with a high mortality mainly because it currently escapes detection at early stages. Identification of an effective biomarker for early detection would improve survival. This study reports documents statistically significant differences in LPL levels between preoperative samples of ovarian cancer patients and those of healthy

controls. The study also confirms that statistically significant elevations in LPL levels are present in patients with early stage disease. Thus, the findings support the utility of LPL, especially LPA, as biomarkers for early detection of ovarian cancer. The study is the first to report significant postoperative changes in specific LPL levels, suggesting that some LPL may also have utility as biomarkers of recurrence. The study also contributes data toward determination of the best combinations of markers and cutoff values for clinical use.

Although our conclusions are still preliminary because our study sample is small and not ideal for demonstrating the value of LPL for screening, our findings regarding the utility of LPL as biomarkers of ovarian cancer are critically important, since the two previous studies showed conflicting results.[8, 10] In order to ensure the validity of our data, only investigators at Moffitt had access to clinical data and the investigators performing LPL measurements at Cleveland Clinic were blinded to the case versus control status of the samples. All statistical analyses were performed at Moffitt.

The reason for the discrepancy between the findings of the two prior studies with interpretable results regarding the utility of LPA as a biomarker for detection of ovarian cancer is unclear. There were many methodologic differences between the two studies, including differences in sample collection, processing and lipid analyses.[8, 10] Our experience suggests that it is critical to maintain consistency of procedures for all samples to be compared, including the time and temperature prior to and during centrifugation, sample storage vials (see below), extraction solvents and methods, establishment of standard curves and mass spectroscopy methods. The following example demonstrates the importance of these aspects. Prior to analyzing the samples included in this report, we analyzed a batch of samples (N=33) that showed lower overall LPL levels than anticipated among both cases and controls, with less

separation than anticipated between levels of cases and those of controls. These findings prompted a review of procedures. Our review identified that the type of microEppendorf tubes used for storage after centrifugation was critically important. If the tubes were not siliconized or prelubricated, as much as 90% of negatively charged LPL were absorbed into the tube walls. Further analysis was performed, including paired storage of identical samples using coated and uncoated tubes, with the resulting differences in LPL levels analyzed. The analysis confirmed that the difference in tubes accounted for the differences in levels observed; therefore data from these samples was not included in the analyses (data not shown). The following suggestions are offered for future investigations of LPL: we recommend using the SafeSeal Microcentrifuge Tubes, Catalog #505-201 (PGC Scientifics, Frederick, MD) for plasma storage, and use of glass ware only (not plastic ware), except for the storage tubes mentioned above.

Further studies are underway to evaluate specificity of LPL measurements obtained not only from healthy controls, but also from women with benign gynecologic disease, other gynecologic cancers and non-gynecologic cancers. Additional studies are planned to evaluate LPL measurements in combination with other markers, including proteomic markers[7] and algorithms of changes in CA 125 values over time.[36] Longitudinal data will allow us to evaluate whether and when specific LPL return to baseline after successful treatment, and their utility in predicting recurrence. Studies are also needed to specifically address the utility of LPL measurements in women at hereditary risk for ovarian cancer, a group in whom early detection is desperately needed, but in whom baseline LPL levels may differ from healthy women at average risk (unpublished preliminary data). Thus, larger studies with the capability of yielding more precise estimates of the sensitivity and specificity of LPL, both alone and in combination with other markers for both screening and detection of recurrence are necessary.

In summary, our findings support the potential of LPL levels as biomarkers of ovarian cancer - specifically LPA levels as diagnostic markers and LPC as prognostic markers. However, these findings require validation in larger studies.

Acknowledgements

This study was supported by an American Cancer Society Clinical Research Training Grant for Junior Faculty (CRTG#-00-196-01-CCE) to Dr. Rebecca Sutphen; additional support was provided by a U.S. Army Medical Research Grant (DAMD 17-99-1-9563) to Dr. Yan Xu. The authors wish to thank JoAnn Runk for administrative assistance; Mrs. Li for technical assistance; Judith A. Betts, R.N. and Jennifer Permuth Wey, M.S. for genetic counseling assistance; Margaret (Peggy) R. Maggiacomo, B.A. and Tricia Holtje, M.S.P.H. for data management assistance; and the patients for everything.

References

1. American Cancer Society I. Cancer Facts & Figures 2003. American Cancer Society, Inc. [Internet]. Available at: http://www.cancer.org/docroots/STT/stt_0.asp. Accessed April, 2003.
2. Mok S.C., Chao J., Skates S., et al. Prostasin, a potential serum marker for ovarian cancer: identification through microarray technology. J Natl Cancer Inst. 93(19):1458-1464, 2001.
3. Schwartz P.E., Taylor K.J. Is early detection of ovarian cancer possible? Ann Med. 27(5):519-528, 1995.
4. Taylor K.J., Schwartz P.E. Screening for early ovarian cancer. Radiology. 192(1):1-10, 1994.
5. Greenlee R.T., Hill-Harmon M.B., Murray T., Thun M. Cancer statistics, 2001. CA Cancer J Clin. 51(1):15-36, 2001.
6. Bast R.C., Jr., Xu F.J., Yu Y.H., Barnhill S., Zhang Z., Mills G.B. CA 125: the past and the future. Int J Biol Markers. 13(4):179-187, 1998.
7. Petricoin E.F., Ardekani A.M., Hitt B.A., et al. Use of proteomic patterns in serum to identify ovarian cancer. Lancet. 359(9306):572-577, 2002.
8. Xu Y., Shen Z., Wiper D.W., et al. Lysophosphatidic acid as a potential biomarker for ovarian and other gynecologic cancers. Jama. 280(8):719-723, 1998.
9. Kim H., Hye-Ran Y., Pyo D. Quantitative Analysis of Lysophosphatidic Acid in Human Plasma by Tandem Mass Spectrometry. Bull. Korean Chem. Soc. 23(8):1139-1143, 2002.
10. Baker D.L., Morrison P., Miller B., et al. Plasma lysophosphatidic acid concentration and ovarian cancer. Jama. 287(23):3081-3082, 2002.

11. Xu Y., Fang X.J., Casey G., Mills G.B. Lysophospholipids activate ovarian and breast cancer cells. Biochem J. 309 (Pt 3):933-940, 1995.

12. Xu Y., Gaudette D.C., Boynton J.D., et al. Characterization of an ovarian cancer activating factor in ascites from ovarian cancer patients. Clin Cancer Res. 1(10):1223-1232, 1995.

13. Gaits F., Salles J.P., Chap H. Dual effect of lysophosphatidic acid on proliferation of glomerular mesangial cells. Kidney Int. 51(4):1022-1027, 1997.

14. Gennero I., Xuereb J.M., Simon M.F., et al. Effects of lysophosphatidic acid on proliferation and cytosolic Ca++ of human adult vascular smooth muscle cells in culture. Thromb Res. 94(5):317-326, 1994.

15. Goetzl E.J., Dolezalova H., Kong Y., Zeng L. Dual mechanisms for lysophospholipid induction of proliferation of human breast carcinoma cells. Cancer Res. 59(18):4732-4737, 1999.

16. Imamura F., Mukai M., Ayaki M., et al. Involvement of small GTPases Rho and Rac in the invasion of rat ascites hepatoma cells. Clin Exp Metastasis. 17(2):141-148, 1999.

17. Genda T., Sakamoto M., Ichida T., et al. Cell motility mediated by rho and Rho-associated protein kinase plays a critical role in intrahepatic metastasis of human hepatocellular carcinoma. Hepatology. 30(4):1027-1036, 1999.

18. Manning T.J., Jr., Parker J.C., Sontheimer H. Role of lysophosphatidic acid and rho in glioma cell motility. Cell Motil Cytoskeleton. 45(3):185-199, 2000.

19. Mukai M., Imamura F., Ayaki M., et al. Inhibition of tumor invasion and metastasis by a novel lysophosphatidic acid (cyclic LPA). Int J Cancer. 81(6):918-922, 1999.

20. Panetti T.S., Chen H., Misenheimer T.M., Getzler S.B., Mosher D.F. Endothelial cell mitogenesis induced by LPA: inhibition by thrombospondin-1 and thrombospondin-2. J Lab Clin Med. 129(2):208-216, 1997.

21. Ren X.D., Kiosses W.B., Schwartz M.A.. Regulation of the small GTP-binding protein Rho by cell adhesion and the cytoskeleton. Embo J. 18(3):578-585, 1999.

22. Sengupta S., Xiao Y., Xu Y. A novel laminin-induced LPA autocrine loop in the migration of ovarian cancer cells. FASEB J. in press. 2003.

23. Xiao Y., Chen Y., Kennedy A.W., Belinson J., Xu Y. Evaluation of plasma lysophospholipids for diagnostic significance using electrospray ionization mass spectrometry (ESI-MS) analyses. Ann N Y Acad Sci. 905:242-259, 2000.

24. Liscovitch M., Cantley L.C. Lipid second messengers. Cell. 77(3):329-334, 1994.

25. Moolenaar W.H., Kranenburg O., Postma F.R., Zondag G.C. Lysophosphatidic acid: G-protein signalling and cellular responses. Curr Opin Cell Biol. 9(2):168-173, 1997.

26. Shen Z., Wu M., Elson P., et al. Fatty acid composition of lysophosphatidic acid and lysophosphatidylinositol in plasma from patients with ovarian cancer and other gynecological diseases. Gynecol Oncol. 83(1):25-30, 2001.

27. Okita M., Gaudette D.C., Mills G.B., Holub B.J. Elevated levels and altered fatty acid composition of plasma lysophosphatidylcholine(lysoPC) in ovarian cancer patients. Int J Cancer. 71(1):31-34, 1997.

28. Van Brocklyn J.R., Lee M.J., Menzeleev R., et al. Dual actions of sphingosine-1-phosphate: extracellular through the Gi-coupled receptor Edg-1 and intracellular to regulate proliferation and survival. J Cell Biol. 142(1):229-240, 1998.

29. Spiegel S., Milstien S. Sphingolipid metabolites: members of a new class of lipid second messengers. J Membr Biol. 146(3):225-237, 1995.

30. Meyer zu Heringdorf D., van Koppen C.J., Jakobs K.H. Molecular diversity of sphingolipid signalling. FEBS Lett. 410(1):34-38, 1997.

31. Spiegel S. Sphingosine 1-phosphate: a prototype of a new class of second messengers. J Leukoc Biol. 65(3):341-344, 1999.

32. Ozols R.F., Rubin S.C., Thomas G., Robboy S. Epithelial Ovarian Cancer. In: Hoskins W.J., Perez C.A., Young R.C., eds. Principles and Principles of Gynecologic Oncology. Philidelphia: Lippincott-Raven Publishers. 958, 1997.

33. Organization W.H. WHO Handbook for Reporting Results of Cancer Treatment. Geneva, Switzerland. 1979.

34. Xiao Y.J., Schwartz B., Washington M., et al. Electrospray ionization mass spectrometry analysis of lysophospholipids in human ascitic fluids: comparison of the lysophospholipid contents in malignant vs nonmalignant ascitic fluids. Anal Biochem. 290(2):302-313, 2001.

35. Zweig M.H., Campbell G. Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 39(4):561-577, 1993.

36. Jacobs I.J., Skates S.J., MacDonald N., et al. Screening for ovarian cancer: a pilot randomised controlled trial. Lancet. 353(9160):1207-1210, 1999.

Figure 1

16:0-LPA/20:4-LPA Levels (μM) in Preoperative Case Samples and Controls

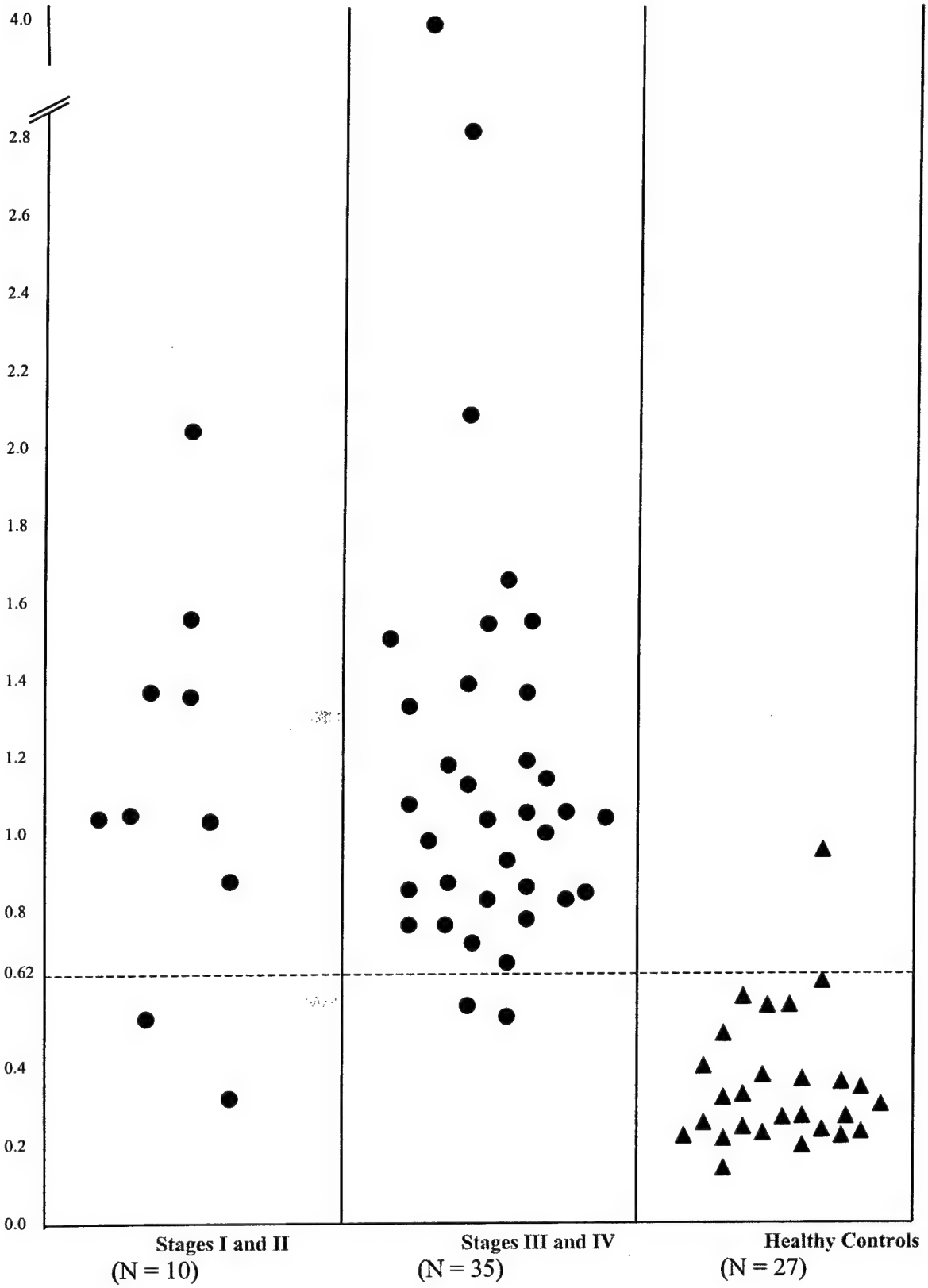


Figure 2

Total LPA Levels (μM) in Preoperative Case Samples and Controls

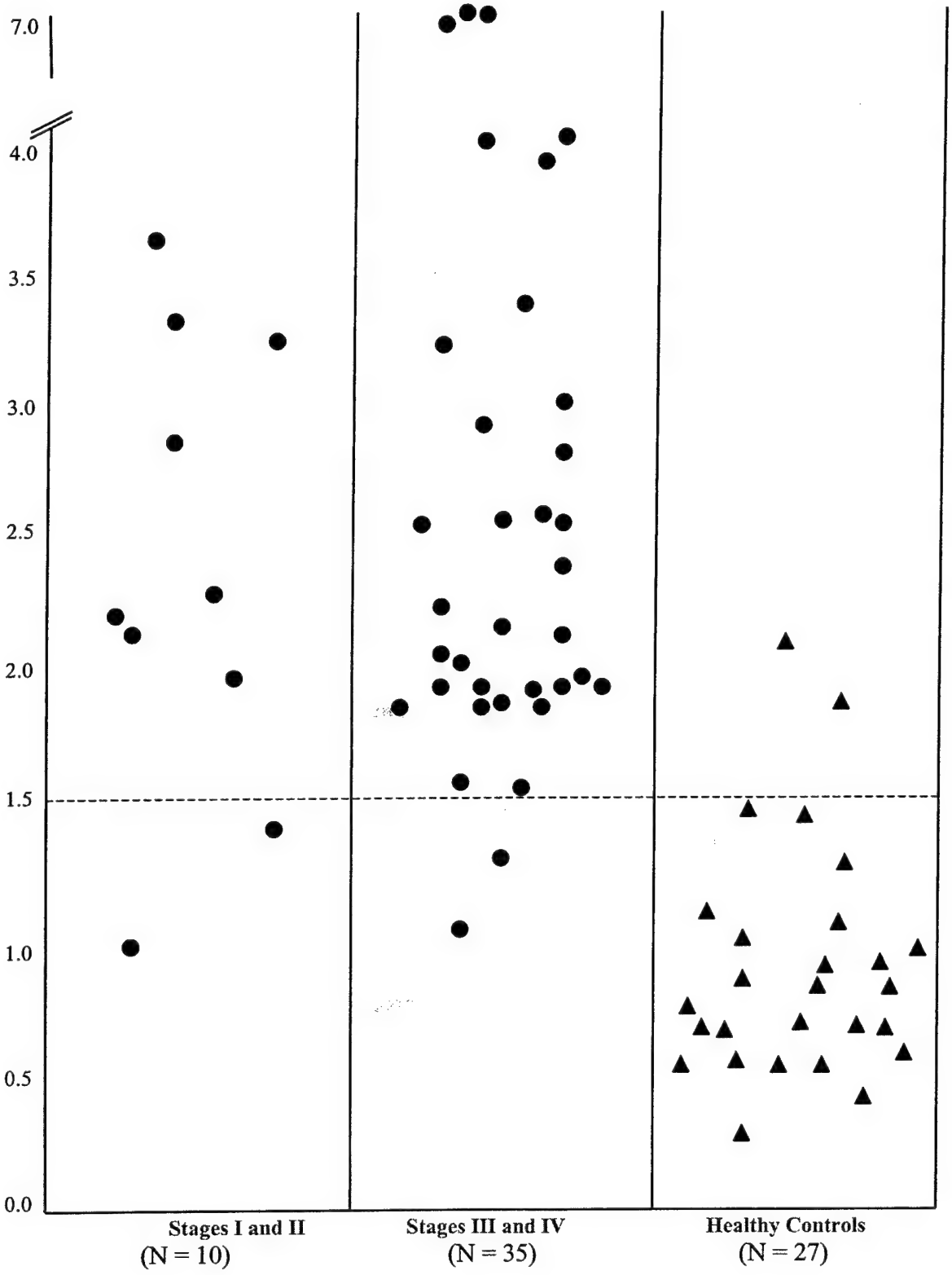


Table 1
Clinical Data for Patients with Ovarian Cancer
(N = 117)

Characteristics	Stages I and II (N = 29)	Stages III and IV (N = 88)
Age, median (range), yr	60 (32 – 77)	59 (26 – 80)
Stages		
I	18 (15.4%)	-
II	11 (9.4%)	-
III	-	74 (63.2%)
IV	-	14 (12.0%)
Grades		
1	10 (8.5%)	11 (9.4%)
2	08 (6.8%)	21 (17.9%)
3	11 (9.4%)	55 (47.0%)
Ungraded	00	01 (0.9%)
Histologic types		
Serous	12 (10.3%)	61 (52.1%)
Endometrioid	11 (9.4%)	07 (6.0%)
Mixed	00	08 (6.8%)
Mucinous	03 (2.6%)	02 (1.7%)
Primary Peritoneal	00	04 (3.4%)
Clear cell	02 (1.7%)	02 (1.7%)
Transitional cell	01 (0.9%)	02 (1.7%)
Brenner	00	02 (1.7%)
Treatment status		
Pre-operative	10 (8.5%)	35 (29.9%)
Post-operative	19 (16.2%)	53 (45.3%)

Table 2

Means (Standard Deviations) for LPL in Controls and Preoperative Case Samples by Stage
(in μM)

Substance	Controls (N = 27)	Stage I (N = 7)	Stage II (N = 3)	Stage III (N = 31)	Stage IV (N = 4)
16:0-LPA ++++	00.14 (00.13)	00.52 (00.39)	00.62 (00.35)	00.73 (00.73)	00.37 (00.14)
18:0-LPA ++++	00.13 (00.10)	00.47 (00.42)	00.29 (00.19)	00.53 (00.51)	00.23 (00.03)
18:1-LPA ++++	00.17 (00.14)	00.37 (00.27)	00.46 (00.29)	00.47 (00.36)	00.32 (00.06)
18:2-LPA ++++	00.16 (00.14)	00.29 (00.26)	00.31 (00.08)	00.46 (00.39)	00.34 (00.09)
20:4-LPA ++++	00.22 (00.16)	00.71 (00.47)	00.31 (00.13)	00.50 (00.31)	00.55 (00.17)
22:6-LPA +++	00.09 (00.07)	00.20 (00.12)	00.16 (00.09)	00.24 (00.24)	00.16 (00.03)
16:0-A-LPA +	00.11 (00.08)	00.15 (00.07)	00.08 (00.05)	00.18 (00.08)	00.19 (00.04)
18:0-A-LPA ++	00.04 (00.06)	00.07 (00.08)	00.10 (00.06)	00.08 (00.06)	00.07 (00.03)
16:0-An-LPA ++++	00.07 (00.05)	00.18 (00.11)	00.11 (00.01)	00.15 (00.10)	00.17 (00.05)
18:0-An-LPA ++++	00.03 (00.04)	00.07 (00.03)	00.11 (00.06)	00.09 (00.07)	00.04 (00.03)
Total A-LPA ++++	00.25 (00.12)	00.48 (00.13)	00.40 (00.10)	00.50 (00.19)	00.47 (00.04)
Total LPA ++++	00.90 (00.43)	02.57 (00.94)	02.15 (00.71)	02.93 (01.77)	01.97 (00.27)
16:0-LPA/20:4-LPA ++++	00.35 (00.17)	01.23 (00.52)	00.92 (00.43)	01.23 (00.70)	00.93 (00.15)
16:0-LPI +++	00.49 (00.47)	00.75 (00.59)	01.88 (01.34)	01.00 (00.64)	00.90 (00.23)
18:0-LPI +++	00.50 (00.43)	00.87 (00.71)	01.77 (02.49)	01.89 (02.05)	00.70 (00.25)
20:4-LPI ++++	00.51 (00.43)	01.35 (00.78)	00.93 (00.95)	01.36 (00.84)	01.36 (00.24)
Total LPI ++++	01.51 (00.79)	02.98 (01.57)	04.58 (02.71)	04.25 (02.81)	02.96 (00.33)
16:0-LPC	52.37 (25.63)	70.65 (30.07)	55.98 (26.57)	52.98 (30.62)	48.10 (21.15)

Table 2 (con't)

Means (Standard Deviations) for LPL in Controls and Preoperative Case Samples by Stage
(in μM)

Substance	Controls (N = 27)	Stage I (N = 7)	Stage II (N = 3)	Stage III (N = 31)	Stage IV (N = 4)
18:0-LPC	15.63 (08.28)	21.00 (09.90)	17.23 (10.98)	14.90 0(9.56)	14.81 (06.57)
18:1-LPC	16.89 (07.27)	21.71 (10.42)	18.97 (13.40)	17.06 (11.40)	17.61 (10.02)
18:2-LPC +	20.21 (07.63)	17.50 (07.72)	16.63 (12.86)	15.12 (08.99)	16.34 (10.36)
20:0-LPC	00.21 (00.07)	00.25 (00.12)	00.19 (00.08)	00.33 (00.41)	00.20 (00.14)
20:4-LPC	10.44 (03.10)	11.60 (04.95)	09.38 (01.56)	10.11 (04.72)	10.36 (03.41)
22:6-LPC +	05.89 (02.24)	10.41 (06.00)	06.98 (04.63)	08.56 (05.96)	09.65 (05.96)
Total LPC	121.65 (47.22)	153.12 (60.02)	125.37 (68.84)	119.07 (64.40)	117.05 (57.06)
S-1-P +++	00.36 (00.27)	00.77 (00.42)	00.50 (00.43)	00.66 (00.48)	00.65 (00.26)

P values show significance levels for differences observed between healthy controls (N = 27) and all ovarian cancer cases for whom preoperative samples were available (N = 45).

\+ $P < .05$
++ $P < .01$
+++ $P < .001$
++++ $P < .0001$

Table 3

Means (Standard Deviations) for Paired Preoperative and Postoperative Samples
(N = 22)

Substance	Preoperative Mean	Postoperative Mean
16:0-LPA	00.85 (00.84)	00.50 (00.28)
18:0-LPA +	00.64 (00.61)	00.33 (00.24)
18:1-LPA	00.55 (00.41)	00.36 (00.29)
18:2-LPA	00.39 (00.43)	00.38 (00.27)
20:4-LPA	00.55 (00.39)	00.47 (00.41)
22:6-LPA +	00.28 (00.28)	00.12 (00.09)
16:0-A-LPA	00.17 (00.09)	00.16 (00.15)
18:0-A-LPA	00.10 (00.06)	00.09 (00.10)
16:0-An-LPA	00.14 (00.07)	00.14 (00.11)
18:0-An-LPA	00.09 (00.07)	00.06 (00.07)
Total A-LPA	00.50 (00.18)	00.44 (00.27)
Total LPA +	03.27 (01.98)	02.16 (01.04)
16:0-LPA/20:4-LPA +	01.41 (00.78)	00.97 (00.51)
16:0-LPI	01.21 (00.91)	01.24 (01.40)
18:0-LPI	02.06 (02.32)	01.28 (01.37)
20:4-LPI	01.38 (00.99)	01.34 (01.06)
Total LPI	04.65 (03.21)	03.86 (02.05)
16:0-LPC	52.61 (30.34)	67.32 (36.06)
18:0-LPC	13.72 (08.62)	18.96 (10.18)

Table 3 (con't)

Means (Standard Deviations) for Paired Preoperative and Postoperative Samples
(N = 22)

Substance	Preoperative Mean	Postoperative Mean
18:1-LPC	15.08 (09.13)	20.95 (10.90)
18:2-LPC ++	13.95 (08.49)	21.67 (07.76)
20:0-LPC +	00.30 (00.43)	00.38 (00.64)
20:4-LPC	09.51 (04.68)	13.19 (05.32)
22:6-LPC	07.64 (05.69)	09.13 (04.77)
Total LPC +	112.81 (59.37)	151.60 (67.52)
S-1-P +	00.78 (00.54)	00.48 (00.29)

Statistically significant differences between preoperative mean values and postoperative mean values are indicated as:

+ $P < .05$
++ $P < .01$

APPENDIX B

Abstract submitted to the annual meeting of the American Society of Human Genetics (October 2002):

Tampa Bay Ovarian Cancer Study – A Population-based Study of BRCA1/2 in Ovarian Cancer.

Tuya Pal, Jeffery P. Krischer, Tricia Holtje, Judith A. Betts, Jenny Permuth Wey, James Fiorica, Edward Grendys, James LaPolla, Hector Arango, Katie Wakeley, Mitchell Hoffman, George Wilbanks, Santo Nicosia, Rebecca Sutphen and the Tampa Bay Ovarian Cancer Coalition

BRCA1 and BRCA2 are believed to account for the majority of hereditary ovarian cancers. Current estimates of mutation likelihood among ovarian cancer patients range from 9.2% (Myriad data) to 11.7% (Ontario population data, the only published population-based data).

To determine the prevalence, spectrum of mutations and genotype/phenotype correlations among ovarian cancer cases, we are conducting a population-based study of unselected incident cases of epithelial ovarian cancer in the geographic regions of Hillsborough and Pinellas counties, Florida (which includes Tampa, St. Petersburg, and Clearwater). Beginning in 2001, we have enrolled 100 women diagnosed with incident ovarian cancer, ascertained through their treating gynecologic oncologists. Medical records and tumor tissue have been reviewed and genetic counseling and DNA testing performed through full sequencing of the BRCA1 and BRCA2 coding regions and adjacent intronic base pairs.

Of the first 100 women enrolled in the study, 15 (15.0%) had mutations in BRCA1 or BRCA2: 7 in BRCA1 and 8 in BRCA2. No mutations were found among the 6 cases with mucinous tumors. No mutations were found among the 5 cases with borderline tumors; thus, the mutation frequency among invasive tumors was 15.8% (15/95).

These data suggest that 1) the frequency of BRCA1 and BRCA2 mutations among invasive ovarian cancer cases may be higher than previously reported, 2) previous studies may have underestimated the contribution of BRCA2 to ovarian cancer, especially mutations outside the ovarian cancer cluster region (OCCR). Preliminary data regarding risk factors, penetrance, associated cancers and tumor characteristics is being analyzed and will also be presented.

APPENDIX C

Abstract submitted to the Frontiers in Cancer Prevention Research annual meeting of the American Association for Cancer Research (October 2003):

Tampa Bay Ovarian Cancer Study: A Population-based Study of BRCA1/2 in Ovarian Cancer

Tuya Pal,[1] Jeffery P. Krischer,[1] Judith A. Betts,[1] Jenny P. Wey,[1] James Fiorica,[1] Edward Grendys,[1] Martin Martino,[1] James LaPolla,[2] Hector Arango,[3] Katie Wakely,[1] Mitchel Hoffman,[3] George Wilbanks,[3] Santo Nicosia,[3] Rebecca Sutphen.[1] Moffitt Cancer Center,[1] Tampa, FL, , Private Practice,[2] St.Petersburg, FL, University of South Florida,[3] Tampa, Florida.

BRCA1 and BRCA2 are believed to account for the majority of hereditary ovarian cancers. Current estimates of mutation likelihood among ovarian cancer patients based on the largest population-based data is 11.7% (Ontario population data). To determine the prevalence, spectrum of mutations and genotype/phenotype correlations among ovarian cancer cases, we are conducting a population-based study of unselected incident cases of epithelial ovarian cancer in the geographic regions of Hillsborough and Pinellas counties, Florida (which includes Tampa, St. Petersburg, and Clearwater). Beginning in 2001, we have enrolled 174 women diagnosed with incident ovarian cancer, ascertained through their treating gynecologic oncologists. Medical records and tumor tissue have been reviewed. Genetic counseling was provided and DNA testing was performed through full sequencing and evaluation for the 5 common large genomic rearrangements. Results are currently available on 164 of these women. Of the first 164 women enrolled in the study, 22 (13.4%) had mutations in BRCA1 or BRCA2: 12 in BRCA1 (7.3% of cases) and 10 in BRCA2 (6.1%). All BRCA1- and BRCA2- associated ovarian cancers were ER-/PR- and ER+/PR+ respectively. Of the BRCA2 mutations, 40% were outside the OCCR region. Most of the tumors had serous histology, and none were mucinous or borderline tumors. The percentages having a positive family history of breast and/or ovarian cancer in a first of second degree relative for BRCA1 carriers (n=12), BRCA2 carriers (n=10), and women with sporadic ovarian cancer (n=142) were 60%, 40% and 30% respectively. The average ages of diagnosis for these 3 groups of women were 54, 59, and 59 respectively.These data suggest that 1) the frequency of BRCA1 and BRCA2 mutations among invasive ovarian cancer cases may be higher than previously reported; 2) previous studies may have underestimated the contribution of BRCA2 to ovarian cancer, especially mutations outside the ovarian cancer cluster region (OCCR); 3) it may be reasonable to offer any woman with an invasive non-mucinous ovarian tumor genetic counseling (up to 15-16% risk in this group); and 4) family history may not be sufficient to accurately predict mutations.

APPENDIX F

Development of the Moffitt Cancer Network

Matt Clark

Moffitt Cancer Network's (MCN)
Matt Clark

INTRODUCTION:
The Moffitt Cancer Network's (MCN) goal is to provide up-to-date oncology related information, resources, and education to oncology health care providers and researchers for the prevention and cure of cancer. Consistent with the aims of the Advanced Cancer Detection Center, the MCN provides access to educational programming, cancer control and clinical protocols, and a mechanism to exchange patient focused information leading to the improved detection and treatment of cancer. The MCN is health care provider focused and complements an array of existing public/lay information sources available elsewhere. It is built around the concept that oncology expertise is geographically centralized, multidisciplinary in nature and of limited availability. The MCN addresses these constraints by increasing availability through a World Wide Web-based design that enables wide access from many geographic locales. The objectives of this project are to:

Collect and organize cancer information to provide educational content to physicians and other health care providers,

Develop and implement software to encode video and audio to enable viewing over the Internet at a range of speeds (bandwidths),

Implement a mechanism to deliver continuing education credits through on-line testing and automated submission/evaluation,

Design and create a web page to permit easy sorting, searching and selection of educational programming,

Design and create a web page to deliver physician referral information that includes submission of an electronic case record consisting of text and imaging data, and

Provide access to case conferencing from remote locations using easily available audio/video to the desktop.

BODY:

Task 1. Collect and organize cancer information to provide educational content to physicians and other health care providers. (Months 1-60).

A schedule of events is determined in coordination with the Moffitt Office of Conference Planning, the Moffitt Multimedia Educational Resources Center, the USF Department of Education, the USF Department of Continuing Medical Education and independent researchers wishing to present. These events include: Grand Rounds, the monthly meeting of the Cancer Control Research Interest Group (CCRIG), Tech Topics (for medical information technology staff), a number of national and local oncology conferences, as well as a number of JCAHO requirements for in-service education for nurses, physicians, and other hospital staff.

The MCN currently has 567 presentations in its library, an increase of 71 from the previous year. Additionally, 16 conferences sponsored by USF and Moffitt are also currently available online.

Schedule videographer coverage of grand rounds and research conferences.
The Network Coordinator, in cooperation with Moffitt Department of Education and the Moffitt Multimedia Educational Research Center, compiles a schedule of for credit events. This schedule is used to determine the scheduling needs of the MCN videographer. The MCN videographer provides audio and video capture of these events digitally and to 90-minute DVCAM (Digital Video Camera) tapes when appropriate.

In late 2002 capture for the MCN migrated to 99% tapeless for routine presentations. Some special events and custom designed instruction are still recorded initially to tape and then digitized for editing. Whenever possible MCN captures presentations electronically without the use of videotape.

Coordinate notification of nursing, pharmacy and other health care providers continuing education presentations.
The Moffitt Department of Education notifies the MCN and all relevant clinical staff of all continuing education presentations and obtains a release from all speakers that permits the distribution of their respective presentation by the MCN. Notifications to clinical staff are multi-modal consisting of e-mails, web postings, and paper fliers. Notification to MCN and other staff required for recording of events is 100% electronic.

Organize the videotaping of faculty scientific presentations for national oncology conferences.
The notification and videotaping of national oncology conferences is scheduled in accordance with the system mentioned above, developed in coordination with the MCN and the Moffitt Education Department. A number of conferences have been added to the MCN library. These presentations are digitized and are made available on the MCN website. The presentations acquired by this activity are codified by a medical librarian, searchable by subject and grouped by their respective conference title.

Coordinate with the Department of Education notification and scheduling of relevant conferences.
The Moffitt Department of Education notifies the MCN of all relevant conferences and the MCN videographer is scheduled in accordance with the videotaping needs of each conference.

Task 2. Develop and implement software to encode video and audio to enable viewing over the Internet in a range of speeds (bandwidths). (Months 1-60)

Explore the application of the Tag development software to support multiple video connections and the impact on network bandwidth.
The MCN has developed a process of digitizing presentations using the Digital Renaissance Tag Composer. Through this process MCN is able to stream presenter's slides and audio simultaneously by using a Synchronized Multimedia Integration Language (SMIL) script file. MCN originally encoded presentation for distribution over ISDN speeds of 128k and modem speeds of 56k. The encoding process used previously created two-network streaming formats, one for ISDN speed connections at 128 kilobytes per second and a second format for current modem technology speeds of 56 kilobytes per second or less. Using the Real media server

software, users linking to a presentation acquire the format (streaming speed) appropriate for their connection bandwidth. The server and the user's player handle this process automatically.

Late in August 2000 MCN determined that the ISDN format was redundant, as it did not offer any significant improvement over the modem format due to the low frame rate of the presentations being developed (sometimes as low as one frame for every three minutes), and MCN has discontinued the encoding an ISDN bit rate media file and thus lowering the production time.

In July of 2000 MCN began to explore the use of the Microsoft Media suite of tools for development of online course content. Microsoft Media provides significant advantages in bandwidth reduction, production and administration time, and potential audience. The MCN has since migrated all processes to Microsoft Media. Windows Media supports a process called Multiple Bit Rate (MBR) video. Put simply, MBR video allows MCN to create a presentation geared toward either low (those users below 128k) or high (those users above 128k) bandwidth. The software determines the minimum speed required by the presentation to stream then negotiates between the client computer (the user) and the server the most bandwidth conserving connection. Using MBR video we are able to stream presentations at 28-32k which previously required 56k+ using Real media. In March of 2000 MCN began the process of converting all assets previously developed in Real media to the Microsoft Media format to better serve our users.

In August of 2001 MCN completed the conversion of all assets to Microsoft Media and began using Windows Media version 7, this provided significant quality improvements over Windows Media version 6.4 while reducing bandwidth requirements.

In 2002 the MCN began using Microsoft Media 8, which provided higher quality at a lower bandwidth than Window Media 7. Compatibility issues between Windows Media 8 and older operating systems forced us to revert back to Windows Media 7. All presentations recorded in Windows Media 8 were re-encoded as Windows Media 7. Since our migration back to Windows Media 7, Microsoft has released Windows Media 9 and stopped supporting Windows Media 8. In mid-2004 MCN will evaluate capturing all new assets in Windows Media 9 or a more recent Windows Media version if one is available and proves backwards compatible.

Evaluate alternative connectivity models, including cable modem connections or access to cable networks as a means to enhance distribution of educational content.

The MCN has evaluated multiple alternative connectivity models, including cable modems, ISDN, ADSL, and traditional T1 & T3 service lines. We have found that cable modems are an excellent method of distributing educational content. Cable modems and ADSL provide a low cost, high bandwidth alternative for the user. This allows educational content to become more dynamic and interactive increasing the quality and effectiveness of the educational activity.

In late 2002 and during 2003 MCN tested these connectivity models for various bandwidth levels of synchronous conventional videoconferencing. Findings show that within the controlled

environment of the state of Florida University Internet connectivity backbone (also connected to Internet2) bandwidths above 256k were capable and exhibited time delays associated with conventional point to point ISDN based conferencing. Tests using TimeWarner's RoadRunner cable modem service in Tampa, Fl proved successful up to 384k. It should be noted however RoadRunner Tampa has a direct connection to the University of South Florida backbone. Tests using high speed DSL (768k) from Arizona to Tampa and Tampa to Tampa where successful and showed no significant transmission quality loss.

Synchronous videoconferencing below 256k was widely successful on most mediums with the exception of the dial-up POTS (Plain Old Telephone System). Both conventional videoconferencing and more recent "Internet only" technologies where explored. Outside of a controlled environment, but with high speed connections (DSL, Cable Modem) low bandwidth videoconferencing is extremely successful and shows expected quality. It should be noted that delay previously associated with Internet and low bandwidth videoconferencing proved not to be significant. Low bandwidth videoconferencing shows to be a viable medium for dissemination of educational content as well as can be used to enchance collaboration and increase productivity between geographically disparate groups.

Evaluate the Internet 2 as to its availability to sustain the necessary bandwidth for the Moffitt Cancer Network.
The MCN has evaluated Internet 2 and found it is ideal mechanism for transporting images, streaming video to and conducting case conferences with other researchers and physicians. Internet 2 is highly effective and we will continue to utilize it when possible.

Resolve firewall and security issues to provide secure communication for clinical data as well as to adequately deal with subscriber/user requirements for security to permit desktop access.
A firewall has been put in place to ensure secure communications for clinical data and to address user security issues. Moffitt IT, in coordination with the MCN has developed a firewall policy relating to streaming media and videoconferencing.

The Moffitt Cancer Center uses key fob technology in conjunction with a secure ID for access to information through the firewall.

Uniform Resource Locator based on specific one-time virtual names.
All prerecorded media is encrypted when necessary and is assigned a unique access requirement for specific use. Users have no direct access to media assets and are provided a virtual link to the assets by a database driven web front end. Additional security methods are still being researched and firewall security is a priority.

Expand the number of Authorized users to the Moffitt Cancer Network.
Expansion of authorized users is critical to the digital convergence with MCN's ongoing research and development. We are now capable of delivering "On-demand", encrypted, and live media to desktops both user specific and publicly when appropriate. In addition, with the recent addition of continuing credit hours for nursing, we have opened a huge medical audience for MCN. It

should be noted that there is no requirement to register or become authorized in order to watch most presentations available on the MCN.

Authorized users increased from 2 to 16 in the year 2000, an increase of 800%.

In mid 2001 a distinction was made between "authorized" and "registered" users. Authorized users are groups of predetermined people who are authorized to view a particular type of content. Registered users are either authorized users who have taken the time to register or non-authorized users who have registered for CME purposes. Authorized, registered users (previously referred to as just "authorized" users) increased from 16 to 68, an increase of 425%, in the year 2001.

Due to recent outreach programs our user base continues to grow. In 2001 the number of authorized, registered users increased from 68-90, an increase of 32%. The number of fully registered users continues to rise at a steady rate. New programs with Moffitt affiliate hospitals established in 2001 generated even greater numbers of authorized, registered users.

Registered, authorized users increased from 90 to 237 in 2002.

Registered, authorized users increased from 237 to 384 in 2003. The number of authorized or registered users reflects only a segment of the utilization of the Moffitt Cancer Network. The overall usage statistics are a more valuable statistic to determine utilization. The statistics (below) show a regular progression in utilization over the past year of the Moffitt Cancer Network. The statistics are separated into internal (users internal to Moffitt Cancer Center) and external (those accessing via the internet). The combined value displays the number of presentations watched and the average number of presentations watched per user. Important to note is the number of sessions (visits) and number of presentations watched per month. The statistics show a regular increase from month to month in site utilization.

MCN Statistics
2002 July to 2003 June
Web Stats

	Jul-02	Aug-02	Sep-02	Oct-02	Nov-02	Dec-02	Jan-03	Feb-03	Mar-03	Apr-03	May-03	Jun-03	Tota
Internal													
Sessions	709	1035	704	689	343	496	325	287	662	374	356	327	63(
Hits	5287	8228	5771	4958	2899	3602	2546	2398	4633	3194	3161	2214	488
H/S Ratio	7.457	7.950	8.197	7.196	8.452	7.262	7.834	8.355	6.998	8.540	8.879	6.771	7.8
Unique Vis	415	620	398	446	186	339	196	146	400	188	183	200	3
S/U Ratio	1.708	1.669	1.769	1.545	1.844	1.463	1.658	1.966	1.655	1.989	1.945	1.635	1.7
External													
Sessions	1771	2253	2180	1977	2027	1934	2513	2119	2458	2210	3051	2048	265
Hits	6020	10086	10891	13497	11646	12473	12321	11862	13598	11345	13005	9521	13626
H/S Ratio	3.399	4.477	0.606	0.652	0.703	0.757	0.815	0.878	0.878	0.945	1.013	1.085	1.3
Unique Vis	887	1129	1197	1116	968	895	1043	1005	1007	1135	1317	1116	10
S/U Ratio	1.997	1.996	0.307	0.331	0.356	0.384	0.413	0.445	0.445	0.479	0.513	0.550	0.6
Combined													
Sessions	2480	3288	2884	2666	2370	2430	2838	2406	3120	2584	3407	2375	328
Hits	11307	18314	16662	18455	14545	16075	14867	14260	18231	14539	16166	11735	1851
H/S Ratio	4.56	5.57	5.78	6.92	6.14	6.62	5.24	5.93	5.84	5.63	4.74	4.94	5.(
Unique Vis	1302	1749	1595	1562	1154	1234	1239	1151	1407	1323	1500	1316	13
S/U Ratio	1.90	1.88	1.81	1.71	2.05	1.97	2.29	2.09	2.22	1.95	2.27	1.80	2.(
Pres Watch	401	674	**650**	**460**	**296**	**365**	**369**	**307**	**467**	**293**	**356**	**252**	48
P/U	0.31	0.39	0.41	0.29	0.26	0.30	0.30	0.27	0.33	0.22	0.24	0.19	0.

H/S Ratio represents amount of time user spends on site per visit
S/U Ratio represents the average number of times users return
P/U Ratio represents number of videos the average user watched that month

Task 3. Implement a mechanism to deliver continuing education credits through on-line testing and automated submission/evaluation. (Months 1-60).

Arrange for automated notification of Department of Education staff for each new presentation selected for the Moffitt Cancer Network.
USF Continuing Professional and Moffitt Conference Planning are notified of each new for-credit presentation selected. Upon successful completion of a CME activity, all relevant information is first stored to our database then is automatically transmitted to USF Continuing Education. The electronic submittal of credit information creates a paperless environment for USF, Moffitt Conference Planning, and MCN. The user is then forwarded to a PDF certificate of completion unique to the individual and activity completed. This further reduces the paper requirement on USF.

Establish ongoing procedures to obtain releases, objectives and CME questions to implement to permit encoding of presentations and inclusion onto the Moffitt Cancer Network.

Presenters sign a release to rebroadcast prior to the videotaping of their presentation. The Moffitt Department of Education works closely with the presenter and the MCN to establish objectives, determine appropriate CME questions and evaluate the overall quality of the educational content of the respective presentation. Upon the completion of this work, all information is passed to the appropriate staff for inclusion into the MCN website for delivery to the user.

Create documentation and procedures to collect appropriate demographics on individuals desiring CME and implement electronic automated notification of our Continuing Education Office to authorize and verify CMEs earned.
Appropriate demographic information is collected from all individuals wishing to receive CME credit for physicians or nurses contact hours. Upon completion of a CME credit or contact hours, the MCN staff is electronically notified. The results of the activity are graded electronically and the information is forwarded to the USF Education Department if a CME credit or contact hour was in fact earned.

In early 2001 MCN developed a process whereby all relevant information pertaining to the educational activity and credit received is transmitted via an encrypted data string directly into the USF Continuing Professional Education certificate-processing cue upon satisfactory completion of credit requirements. This eliminates a number of steps while reducing the probability of error. MCN currently uses a redundant system whereby USF Continuing Education records are audited periodically against MCN records to ensure proper certificate issuance.

Automatically link the Cancer Library to the acquisition process so that they are aware of new acquisitions and receive opportunities to extract key words for indexing, sorting and searching.
Upon the completion of the digitization of a presentation, the digitized presentation is forwarded to the Cancer Center Librarian for review. The Cancer Center Librarian extracts key words used for indexing, sorting and searching presentations on the MCN website. These keywords are added to the MCN website database for each respective presentation.

In late 2002 this became an automated process whereby the cancer center librarian is automatically notified when an event is added to the MCN. The librarian may then go to a keyword management system to enter data. The keyword management system is sortable and searchable and allows the library to get a snapshot of the state of the keyword database by seeing which presentations have been keyworded and which have not.

Extend the CME process to include CEUs for nursing and pharmacy.
The MCN currently offers CME credit for physicians as well as contact hours for nursing continuing professional education (CEU). The certifications are provided in cooperation with the USF College of Medicine and Nursing, respectively. We are continuing to explore the applicability of the content to other healthcare providers, such as pharmacists, and the requirements to offer continuing education credits.

In late 2002, the MCN began to issue Pathology certifications that have been highly utilized.

Expand the educational content offerings to include mandatory requirements for risk analysis, HIV, infection control, etc.
The MCN has expanded the educational offerings to include a number of JCAHO requirements for nurses, physicians and staff. These offerings are available internally to all personnel via the Moffitt Cancer Center Intranet. Major mandatory education such as Domestic Violence, HIV/AIDS, and Bioterrorism are available to external users as well.

MCN is now in the process of making available educational material regarding open clinical trials in addition to the topics mentioned above.

Task 4. Design and create a web page to permit easy sorting, searching and selection of educational programming. (Months 1-24)

Organize educational content along primary audience lines and develop a key word searching algorithm to subset for presentations.
An algorithm has been developed allowing keyword searching. The keywords are assigned during the review of the presentation by the cancer center library. A new algorithm was developed in 2001 allowing a more efficient search. The MCN website provides chronological ascending/descending, keyword search, search within results, and presenter last name, first name searches.

Implement a database for key words according to a standard nomenclature, utilizing NLM MeSH headings, cancer site, etc.
A keyword database has been created and is used by the MCN website for searching. The keywords are determined by the Cancer Center Librarian prior to the addition of a presentation to the MCN. The keywords are based on NLM MeSH standards.

In late 2001 the MCN began to track user searches to gain a better understanding of how users search the website. The information gathered has led us to add not only MESH keywords, but layperson keywords as well.

Expand implementation of Active Server Page (ASP) extensions to the multimedia hypertext (HTML) by adding onto the 'back-end' of the Web application i.) procedural language scripting and ii.) the ability to exchange information with a fully functioning database.
ASP has been used throughout the site to produce dynamic, database driven web pages. ASP is used in all areas of the site to set procedural paths, increase security and generate dynamic content from the MCN databases.

Expand and refine the JET database to incorporate user defined search phrases that are located within a variety of fields associated with the database, including a textual 'objectives' section, MeSH headings, cancer site, canned search categories, etc.
The MCN has increased the capability of the Jet database to allow user defined search phrases. These phrases search for matches in the textual 'objectives' section, MeSh headings (keywords), cancer site, and canned search categories.

Monitor utilization by remote site to evaluate the frequency and demand for various types of educational content to permit refinements and revisions to improve offerings.
The MCN gathers extensive information in regards to use of the MCN website. This information includes website traffic, which asset was accessed, time spent, keywords searched for, the number of presentations watched, for credit or not, and the frequency with which each presentation is watched.

Task 5. Design and create a web page to deliver physician referral information that includes submission electronic case record consisting of text and imaging data. (Months 1-36)

Develop and implement a database to archive text and imaging data for retrieval by consulting Cancer Center physicians and integration with Moffitt Cancer Center clinical information systems.
Moffitt has a DICOM server which, when combined with secure Internet protocols, may be used to transmit and receive DICOM-compliant images to and from partners on the Internet. These images are securely relayed to and from Moffitt's PACS viewing stations. This technology has been proven to work in experiments with the Haley Veterans' Administration Hospital and Cornell University. Radiology is currently working with Morton Plant Hospital to develop a permanent, Internet-based method of exchanging patient radiographic images. This system is currently used for all patients of Moffitt.

Develop a structured computerized clinical case description that provides a minimally relevant set of data that describes a clinical case for second opinion and consultation.
Efforts to date have focused on image transfers and the capability to be DICOM compliant. Appropriate mechanisms have been developed along with interfaces to hospital PACS and Radiology Departments.

Additional efforts focus on Clinical Genetic electronic pedigrees for use in remote case conferencing. This is expected to expand greatly in the coming year as the use of telemedicine for clinical genetics increases.

Acquire hardware and software to provide audio and video real time and time shifted streaming of case conferencing to remote locations for user viewing over secure communication links.
In July 2000 MCN procured rack mounted dual processor servers and audio/video equipment for the purpose of providing both real-time streaming of media as well as simultaneous capture of that media for archive.

In December 2000 MCN began exploring the use of low-cost, low bandwidth one-way and two-way case-conferencing equipment. This equipment would allow the patient to contact and conference with their respective physician without leaving their home. A preliminary trial of the equipment and its functionality, conducted in 2001, was successful.

In October 2001 MCN began streaming a monthly genetics case conference to our affiliate hospitals.

In 2002 MCN evaluated the use of a synchronous conferencing system called Lotus Sametime for the monthly genetics case conference. We believe this will improve the efficacy of the conference and by allowing remote users to interact with the geneticist.

The Lotus Sametime system along with the Microsoft Windows Media system has been extended not only into clinical genetics but also into various large scale international research programs.

Establish the necessary gateways and bridges to provide connections at a range of bandwidths to support remote connectivity.
All processes are controlled remotely and designed for live to archive times of no more than five minutes. In other words, five minutes after a live broadcast event is completed, an "On-Demand" rebroadcast will be available to specific users. The former being broadcast via secure port and virtual link and the latter are encrypted for use with a specific key.

The Lotus Sametime System implemented in 2002 acts as gateway for limited traffic. A gatekeeper has been established to handle all other IP based traffic. These support bandwidths range from 32k to 3MB per second.

Design and implement web-based front ends to Moffitt Cancer Center clinical systems to permit secure access to patient information of patient's referred or submitted to case conferencing or second opinions.
Moffitt is in the process of a Cerner Clinical record system implementation. Upon the completion of this project, restricted access to case information could be made available via the web.

Task 6. Provide access to case conferencing from remote locations using easily available audio/video to the desktop. (Months 1-48)

Complete telegenetics experiment to assess feasibility and acceptability of this format for the exchange of clinical information.
The telegenetics experiment has been completed. Findings are as follows:

Of 74 eligible subjects, 60 agreed to participate. There were no differences in previous technology exposure between the 14 who declined participation and the 60 who agreed. Of the 60 participants, 30 received their initial genetic counseling session via telemedicine, and 30 received the session face-to-face. There were no differences perceived in patient or provider satisfaction between the face-to-face and telemedicine pre-test sessions. Of the 60 participants, 23 proceeded with genetic testing, 12 of whom had received the initial session face-to-face and 11 via telemedicine. Based on the crossover design of the study, these 23 individuals received their post-testing genetic counseling session via the opposite method from their initial session. A two-sided test of significance showed no difference in overall satisfaction between telemedicine and face-to-face sessions.

A new telegenetics study is being designed to be submitted to the NCI National Community Clinical Oncology Program . This study is intended to explore the feasibility of delivering cancer

susceptibility genetic counseling and testing services via telemedicine. We anticipate randomizing 200 participants.

Each individual who presents for cancer genetic counseling (having been referred through their health care provider or through self-referral) will be recruited for the study, until study accrual has been completed.

Each individual will be asked to participate in the study by agreeing to be randomized to receive genetic counseling via one of two methods 1) face-to-face (standard) or 2) telemedicine. As part of the study explanation, candidates will be provided with an introduction to telemedicine by physically seeing the equipment and being allowed to "try it out" by briefly visualizing and talking with another individual via the connection. This "hands-on" introduction should ensure informed consent for the study. Patients who elect to participate in the study will sign a written informed consent form.

All individuals who have agreed to be randomized for the study will be asked to complete the State-Trait Anxiety Inventory evaluating their pre-test genetic counseling anxiety levels. This serves as a baseline and is administered after an individual is ascertained to be at increased risk for familiar cancer, but before the individual has undergone genetic counseling.

A BRCA1 & 2 knowledge questionnaire consisting of 11 true-false measures, which include items used in core instrument was developed for use by the National Center for Genome Research (CHGR) Cancer Studies Consortium. This instrument will be used to assess differences in knowledge transfer between the two delivery modalities.

One week after the conclusion of the post-test genetic counseling session, the Strait-Trait Anxiety Inventory and the knowledge instrument will be administered to the patient via telephone to assess differences in anxiety levels that may exist between the face-to-face and telemedicine consultations.

Implement additional sites to expand this program and resolve billing issues within the context of existing laws and regulations regarding telehealth and teleconsultation programs.
A preliminary structure has been put in place for support, however legal limitations existing within the state hinder rapid progress on developing a large scale clinical program. New legislation is expected during the next two years and we are working with other institutions to address billing, reimbursement and practice issues.

The genetics department has extended the reach of the genetics case conferencing program into ten statewide centers and continues to explore adding case conference members.

Establish the necessary gateways and bridges to provide connections at a range of bandwidths to support remote connectivity.
MCN is implementing two strategies, traditional videoconferencing and Sametime web based videoconferencing at the desktop. Sametime provides a low cost, secure mode of communication, primarily aimed at researchers and M.D.s involved in case conferencing.

Traditional videoconferencing provides a widely adopted videoconferencing modality and therefore use of the equipment does not necessarily mean the adoption of new technology for remote centers.

Develop tunneling or other secure links to resolve firewall issues regarding LAN configurations at both the Moffitt Cancer Center and remote sites.
Moffitt is using Virtual Private Networks with key fob and biometric authentication technology. A Cisco Multimedia Conferencing Server is being placed in tandem with the firewall to provide a secure single point of access through the firewall for videoconferencing.

Acquire and install technology in conference centers where case conferencing generally occurs for selected clinics to permit retrieval and display of multiple images and clinical data submitted for this purpose by remote users.
For each site, a detailed plan of operations has been developed to establish the capability to schedule and transmit signals for MCN distribution. MCN has implemented streaming equipment for Clinical and Research presentations given at the center.

MCN technology has been applied to the conferencing centers in the new Moffitt Clinic and Research buildings. The buildings are equipped to allow for automated capture of educational assets from any conference room within the facility. The cost of this was absorbed as part of the overall construction cost to Moffitt. Money has been budgeted in the current year to bring a percentage of the non-MCN equipped conference rooms up to the same standards as the newly equipped room in the new facilities.

In March 2001, MCN successfully completed the installation of case conferencing equipment in two primary conference centers in the main building and the original research center building.

Plans for a fully interactive case conferencing center are in progress. The center would provide access to digital radiology, relevant patient information, and a host of other technologies.

Assess utilization of this technology to refine and revise formats and improve the quality and ease of remote access.
MCN has made it a priority to improve the quality of its products. Moving towards the use of Microsoft products and its MPEG-4 streaming format has reduced labor and increased quality across the board. MCN has implemented programs for remote control of streaming servers. A migration to XML took place in June of 2002, improving portability of the system. The use of scan converters in capture of educational content has greatly improved quality of the final asset. Finally, changes in its business practices have reduced labor requirements and increased quality and functionality as well as increased the customer base.

MCN continues to work to increase capability and functionality, improving video quality, and lowering bandwidth requirements for the user, while at the same accruing more content and reducing the production time by streamlining and automating the process.

Sametime videoconferencing was implemented to provide low cost case conferencing access to smaller, possibly rural medical centers.

The MCN outfitted conferencing facilities in the newly constructed buildings to produce an end-to-end digital image and greatly increases the overall quality of the assets produced.

KEY RESEARCH ACCOMPLISHMENTS:

- The Moffitt Cancer Network is available to users and can be found at http://network.moffitt.usf.edu
- The MCN currently has 576 presentations in its library. Additionally, 16 conferences sponsored by USF and Moffitt are also currently available online.
- All approved Grand Rounds presentations have been taped by the Moffitt Multimedia Education Resources Center (MERC) for over two year preceding this report. The video was previously captured on digital DVCAM 94 minute tapes. Currently we are running in a tape-less environment.
- Since many of the presenters use only 35mm slide for their presentations, a process of creating final production audio/video Real media for streaming via TCP/IP has been developed. This process requires post-production labor and requires the best of the video's individual frames to be captured a second time to recreate higher quality computer images. MCN has made significant progress in this area and as of June 2000 has begun using presenter's PowerPoint files when ever possible to bypass the second image rendering process. This has reduced labor time from 3.5 days to about 5 hours, while increasing image quality noticeably. This labor savings is not realized when presenters are using 35mm film only. This methodology was modified to capture slides, overheads and computer screens digitally without a camera. The new methodology has reduced post-production time to virtually nothing. This allows us to concentrate on acquisition of new material.
- In addition to pre-presentation file acquisition, MCN has begun the development of a presenter packet. When finished, this packet will inform presenters to repeat important questions asked at the end of events like Grand Rounds and these will be added to the content to be available to medical professionals at the MCN website.
- National oncology conferences have been taped and included in the MCN website database.
- Conferences have been subdivided into their respective presentations and are categorized searchable as well as searchable using the website database Access Jet engine. All conferences are pre-qualified for their ability to become online educational materials by the University of South Florida College of Medicine and, more recently, the University of South Florida College of Nursing.
- MCN began simultaneous live streaming and archiving in late 2001. This process greatly reduces postproduction time while increasing access to live events.
- MCN has completed the move to camera-less and tape-less acquisition of presentations using a host of digital equipment.

REPORTABLE OUTCOMES:

- Patents and licenses applied for and/or issued;

A notice of disclosure has been filed with the USF office of patents in anticipation of the completion of a patent application.

•Presentations

- The Moffitt Cancer Network Vision, Jeffrey Krischer, Ph.D. April 2001
- The Moffitt Cancer Network, Lessons Learned and New Directions, Matthew Clark, B.S. October 2001
- The Moffitt Cancer Network 2002, Matthew Clark, B.S. April 2002
- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., May 19, 2002 Medical Library Association Conference, Dallas TX
- No-latency video architecture, efficiency and a new tomorrow for on-line education, Matthew Clark, B.S. June 2002
- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., June 19, 2002 Tech Topics, Moffitt
- Keyword Indexing: Adding Value to the Moffitt Cancer Network [MCN] Web-based Education, Sue Felber, M.S., October 19, 2002 Southern Chapter, Medical Library Association
- Disseminating Library Instruction to the Desktop via the Web, Sue Felber, M.S., October 19, 2002 Southern Chapter, Medical Library Association
- Telemedicine Today and Tomorrow, Matthew Clark, B.S. October 2002

•Abstracts

- J Permuth-Wey, JA Betts, AB Cantor, JP Krischer, R Sutphen: Cancer Genetic Counseling and Testing by Telemedicine - Results of a Feasibility Study (Abstract). American Journal of Human Genetics (2002) 71(4): 343.

CONCLUSIONS:

The purpose of this research is to create processes that allow medical professional to extend their abilities through the use of electronic media. MCN has evolved in pace with the change of that technology and because of its foresight and its dedication to purpose it has kept ahead of the technology. MCN has realized that streaming media processes are now capable of high definition presentations at low bandwidths and has developed the best possible processes for producing usable educational media delivery using network technology. MCN's research into these processes has revealed the need for specific products and their uses. Several new programs have been developed to address these processes. For example, to cut down on the need for many new employees, MCN has developed a broadcast program that will allow a single user to set start/stop times on a given event at a given location.

Conventional videoconferencing has limitations of cost and support while not meeting security and privacy requirements of HIPAA. Lotus Sametime may be a cost effective means of HIPAA complaint case and video conferencing. Providing second opinion and expert information to referring physicians is an extremely important piece of MCN's research. While continuing education is a given, in the final analysis, it may be in the medical professional interaction that MCN becomes most useful. If it were determined effective Sametime would provide a secure,

cost effective case conferencing system that would allow smaller and rural centers as well as individual doctors a means to gain access to Moffitt expertise.

This experience has led to the demonstration of efficient and effective web-based video-conferencing methodologies. The environment regarding the presentation of lectures, case conferences and lecture programs has proven effective and well accepted by health care providers. Future plans have led to the integration of this technology in other research programs facilitating education, communication and data sharing. These applications have built upon the technology base developed by the MCN and have led to independent funding to support specific research projects.

REFERENCES: None

a